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Localization of α-Tocopherol Transfer Protein in Trophoblast, Fetal Capillaries' Endothelium and Amnion Epithelium of Human Term Placenta

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Vitamin E has been linked to fertility since its discovery in 1922. However, the exact mechanism by which α-tocopherol allows pregnancy to continue until term has remained puzzling over the last 80 years. α-Tocopherol transfer protein (TTPA) is expressed in liver and in Purkinje cells of the cerebellum. TTPA is suggested to be responsible for the transfer of α-tocopherol across barrier membranes. *Ttpa*-knockout mice are infertile and show symptoms similar to those observed in severe vitamin E deficiency. We thus investigated TTPA expression in human placenta and whether clues from its localization in different parts of the placenta might be of functional significance. *TTPA*-mRNA transcripts were quantified with a fluorescent 5′-nuclease assay (TaqMan®) in five different tissues. Placental expression ranged second behind that of liver. Immunohistochemistry identified TTPA in the cytosol but also in nuclei of the trophoblast and in the endothelium of the fetal capillaries. Expression in trophoblast and fetal capillaries' endothelium indicates a role of TTPA in the stereoselective transport of *RRR*-α-tocopherol from the maternal to the fetal plasma. In amnion epithelial cells, however, TTPA was predominantly located in the nuclei. Nuclear localization of the protein may represent a novel function of TTPA.

Keywords: α-Tocopherol transfer protein; Vitamin E; Placenta; Trophoblast; Amnion

Abbreviations: AA, amino acid; Ct, threshold cycle value; HPRT, hypoxanthine phosphoribosyltransferase 1; IPTG, isopropyl β-D-1-thiogalactopyranoside; PCR, polymerase chain reaction; PGE_2, prostaglandin E_2; *RRR*-α-tocopherol, naturally occurring stereo-isomer of α-tocopherol; TAP, tocopherol-associated protein; TTPA, α-tocopherol transfer protein; VLDL, very low-density lipoprotein; VLDLR, very low-density lipoprotein receptor

INTRODUCTION

Vitamin E (α-tocopherol) is linked to animal fertility ever since the seminal work of Evans and Bishop (1922), who discovered this essential factor for animal reproduction. Female rats fed on a vitamin E free diet are sterile or resorb their fetuses. These phenomena can be reversed by administration of pure vitamin E to pregnant animals.[1,2] Thus, the name α-tocopherol is derived from τοκοσ (tokos, Greek for "childbirth") and φερειν (pherein, Greek for "to bring forward").[3] Impaired fertility is also observed in animals lacking the α-tocopherol transfer protein (TTPA). The resorption of their fetuses in mid-pregnancy by homozygous *Ttpa*-knockout mice can be corrected by very high α-tocopherol supplementation.[4,5] The exact mechanism by which α-tocopherol allows pregnancy to proceed until term has remained puzzling since its discovery. The effect of homozygous *TTPA*-mutations on human fertility is unknown.

TTPA specifically sorts out α-tocopherol from all incoming tocopherols and tocotrienols for its incorporation into very low-density lipoprotein

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(VLDL) in the liver and for further transportation and distribution among tissues. It is also responsible for the stereo-selective incorporation of the "natural" stereo-isomer *RRR*-α-tocopherol into VLDL. Absence of TTPA leads to the rapid disappearance of α-tocopherol from plasma.[6] Patients with mutations in the *TTPA*-gene have very low to undetectable plasma α-tocopherol levels and must be on livelong supplementation with high dosages of vitamin E in order to prevent severe damage to the central nervous system including characteristic ataxia.[7,8] α-Tocopherol plasma levels in human fetuses are generally lower than those in their mothers, but levels rise towards the end of pregnancy.[9,10] The stereo-selective uptake of α-tocopherol from the maternal plasma by the fetus hints to an active transport system. Acuff *et al.* (1998) demonstrated with differently deuterated *RRR*- and *all-rac*-α-tocopherols that *RRR*-α-tocopherol is enriched in humans by a factor of 3.42 at the passage through the placenta indicating a specific transport against a concentration gradient.[11] Gordon *et al.* (1996) identified two [^{3}H]α-tocopherol binding proteins in the cytosol of the human placenta.[12] These findings led us to assume that TTPA might be present in human placenta despite the fact that TTPA had not been detected in mouse placenta at any time during development.[5] We therefore investigated *TTPA*-expression and localization in the human placenta to get hints to the putative function of vitamin E in pregnancy.

MATERIAL AND METHODS

Materials

The following materials were obtained from commercial sources: custom peptide synthesis, coupling to KLH and immunization of animals (Pineda Antikörperservice, Berlin, Germany); formaldehyde, ethanol, *n*-hexane, β-mercaptoethanol, IPTG, Entellan® (Merck, Darmstadt, Germany); TRIzol®, Superscript® III reverse transcriptase, Platinum® Taq, Western Breeze®-kit (Invitrogen, Karlsruhe, Germany); custom primer and TaqMan®-probe synthesis (TibMolbiol, Berlin, Germany); pGEM-T-vector (Promega, Mannheim, Germany); *Pfu*-DNA-Polymerase (Stratagene, Heidelberg, Germany); IMPACT-CN®-System (New England Biolabs, Frankfurt, Germany); Centricon® YM-10 centrifugal filter units (Millipore, Schwalbach, Germany); Histoplast®, Hypercenter®XP (Shandon, Pittsburgh, PA, USA); chemicals for immunohistochemistry if not stated otherwise (DAKO, Hamburg, Germany); SUPERFROST® adhesive microscope slides (Erie, Portsmouth, NH, USA), anti-cytorkeratin 7 antibody, Hydromount® (Vogel, Giessen, Germany), anti-human cytokeratin 7 antibody, mouse (DAKO, Hamburg, Germany), biotin-SP-conjugated AffiniPure® donkey anti-rabbit IgG, FITC-conjugated AffiniPure® goat anti-mouse IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA), Streptavidin Cy3 Conjugate, Hoechst 33258 (Sigma, St. Louis, MO, USA).

Preparation of Placental Tissues

After written informed consent according to the Declaration of Helsinki tissue samples were obtained from the chorionic and basal plates as well as from the villous tissue of five term placentae of healthy pregnant women at term within 30 min of vaginal delivery. Part of the tissue was immediately flash frozen in liquid nitrogen for later RNA extraction and measurement of placental α-tocopherol levels. Samples for immunohistochemistry were immediately fixed in 4.5% formaldehyde for 24 h.

Preparation of Other Control Tissues for RT-PCR

The mRNA from fresh control tissues was obtained after written informed consent of the patients or their guardians according to the Declaration of Helsinki. The liver samples ($n = 6$) were cut from the non-tumorous rim of excised liver tumors. The muscle samples ($n = 5$) were attained after orthopedic surgery. The cultured skin fibroblasts ($n = 5$) were from diagnostic samples of siblings of children with metabolic diseases, that turned out to be normal. The Epstein–Barr Virus immortalized lymphoblastoid cells ($n = 6$) were from healthy adult volunteers. All tissues were flash-frozen in liquid nitrogen right after excision or harvesting the cell culture.

Measurement of α-Tocopherol in Placental Tissue

Villous placental tissue was grounded in liquid nitrogen and a 1:80 (w/v) homogenate was prepared with phosphate buffered saline and butylhydroxytoluol was added (57 mM in ethanol, 2 μl/mg tissue). α-Tocopherol measurements were performed in duplicate in 100 μl homogenates (1:80, w/v) after addition of 20 μl laurylsulfate (0.1 M) using HPLC with electrochemical detection and internal standardization as previously described.[13] The results were given as nanomole α-tocopherol per gram placental protein.

Quantitative RT-PCR

RNA was prepared from homogenized tissue samples with TRIzol®-reagent and reversely transcribed at 42°C with Superscript® III reverse transcriptase according to the manufacturer's

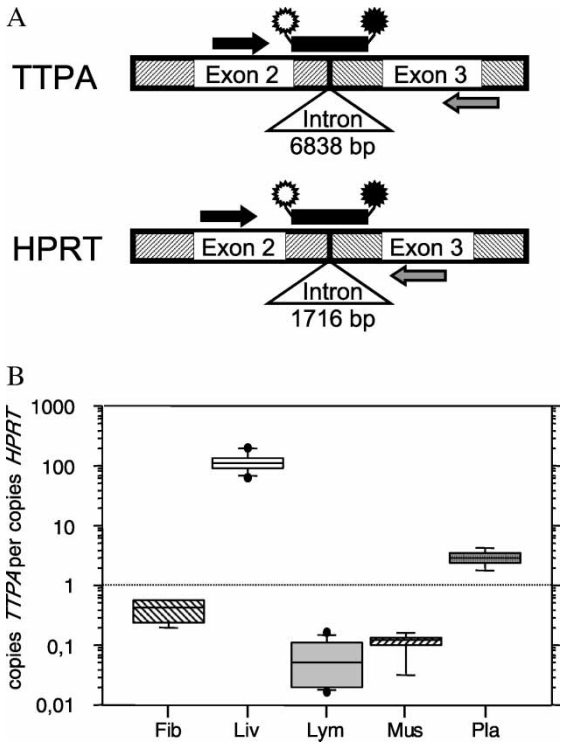

FIGURE 1 (A) Position of the TaqMan®-probe and the forward and reverse primers for quantification of *TTPA*- and *HPRT*-mRNA transcripts from human tissue. In order to prevent amplification of genomic DNA the PCR-primers are placed in two adjacent exons separated by a large intron. Additionally, the TaqMan®-probe is located on the exon/exon-boundary. (B) Quantification of *TTPA*-mRNA transcripts in relation to the transcript number of the "house-keeping" gene *HPRT*. The transcript numbers in liver were highest followed by placenta. The box-plots depict the median, the interquartile and interdecile ranges. The most extreme outliers are depicted as black dots. Fib, fibroblasts; Liv, liver; Lym, lymphoblastoid cells; Mus, muscle; Pla, placenta.

instructions. Quantification of *TTPA*-cDNA transcripts was performed by a fluorescent 5′-nuclease assay using the TaqMan®-chemistry (Applied Biosystems, Weiterstadt, Germany).[14] In order to prevent amplification of genomic DNA, the TaqMan®-system for *TTPA* (GenBank NM_000370) was designed in a way that the forward 5′-TGG AGT CCT GAG ATC CAG GG-3′ (S416) and the reverse 5′-CCG CTG AGT TTC TAC CTC CTG T-3′ (S417) primers lay on opposite sides of a large intron of 6838 bp. Additionally, the TaqMan®-probe FAM-ATT TAC AGA ATC GCA CAC TGG GAC CCC-X-TAMRA (S418) was designed to hybridize on the exon/exon boundary. Thus, the probe cannot hybridize to a PCR-product generated from genomic DNA (Fig. 1A). For quantification, an internal standard was prepared of the PCR-product S416–S417 cloned into the pGEM-T vector. A calibration series of plasmids ranging from 10^7 to 1 copy/μl was prepared. The PCR was run on the ABI PRISM 7700 Sequence Detection System (Applied Biosystems) with a hotstart Taq-polymerase. A 10-min denaturation step at 94°C was followed by 45 cycles of denaturation at 94°C for 30 s and annealing/extension at 67°C for 60 s.

RT-PCR results are difficult to compare between mRNA-preparations from different samples unless the results are normalized to a ubiquitously present "house-keeping" gene, which has to be amplified in parallel. In the present experiment we choose the *Hypoxanthine Phosphoribosyltransferase 1* (*HPRT*)-gene (GenBank NM_000194). The considerations on the design of the *HPRT* TaqMan®-system followed the lines described above. The position of the forward 5′-GAG GAT TTG GAA AGG GTG TTT ATT C-3′ (S556) and the reverse 5′-ACA ATG TGA TGG CCT CCC A-3′ (S557) primers as well as the TaqMan®-probe FAM-CAT GGA CTA ATT ATG GAC AGG ACT GAA CGT CTT G-X-TAMRA (S558) are depicted in Fig. 1A. The TaqMan®-readings were analyzed with the "Sequence Detector v1.6.3" software (Applied Biosystems) and related to the readings of the calibration curve. We correlated each sample of the calibrator with the threshold cycle value (Ct). The Ct-value corresponds to the cycle number after which the sample becomes positive. A positive signal was defined as a measured fluorescence >10 SD above the background fluorescence of 5,6-carboxy-x-rhodamine.

Recombinant Expression of Human TTPA in *Escherichia coli*

The open reading frame of the *TTPA* was amplified with the forward primer 5′-*GGT GGT TGC TCT TCC AAC* ATG GCA GAG GCG CGA TCC C-3′ (S411) and the reverse primer 5′-*TCG TTG CGG CCG* CAG TTA GGA ACG CAT TCA CAT GAC ATA AC-3′ (S403) from a liver cDNA preparation with "proof-reading" *Pfu*-DNA polymerase. S411 has a 5′-overhang with an engineered *Sap* I and S403 with a *Not* I restriction endonuclease site (overhangs are depicted in italics). This way the PCR-product could be cloned into the *Sap* I-*Not* I multiple cloning site of the vector pTYP11 of the IMPACT-CN® system. pTYP11 creates a fusion protein between an N-terminal intein tag in which a chitin binding domain allows affinity purification of the fusion precursor on a chitin column. After induction of the transfected *Escherichia coli* (ER2566) culture at $OD_{600} = 0.8$ with 0.5 mM IPTG, it was incubated at 16°C for 18 h. The pellet was harvested, sonicated and the supernatant was applied on a chitin column. After a short wash with 50 mM β-mercaptoethanol the column was left at 4°C for 24 h. In the presence of β-mercaptoethanol the intein undergoes specific self-cleavage thus releasing the TTPA-protein without any appendages at its N- or C-terminus. After elution of the protein it was washed and concentrated with Centricon® YM-10 centrifugal filter units. We verified the identity of the resulting TTPA-protein by Western-blotting and

additionally by peptide mass fingerprinting via trypsin digestion and subsequent MALDI-TOF mass spectrometry (data not shown).

Generation of Antibodies against Human TTPA

N-terminal (NH_2-MAEARSQPSAGPQLNALPDH-COOH, AA#1–20 of *Homo sapiens*, GenBank NM_000370) and C-terminal (NH_2-DILPREYGGK-EFSME-COOH, AA#239–253 of *Mus musculus*, GenBank XP_131274, the mismatches between human and mouse are underlined) peptides were synthesized and coupled with keyhole limpet hemocyanin as described previously.[15] Two rabbits were immunized with each conjugate subcutaneously and boosted at days 20, 30, 40, 61, 75, 90 and 120. Serum was obtained thereafter and tested on a Western-blot with 40 μg human and mouse liver homogenate. At serum-dilutions between 1:1,000 and 1:10,000 of the N-terminal antibody a single band was detected at around 32 kDa at the human tissue with a secondary alkaline–phosphatase-conjugated anti-rabbit IgG-antibody and subsequent chemiluminescent detection (Western Breeze®). No satisfactory result was obtained with mouse liver homogenate. The C-terminal antibody was also tested on human liver homogenates and on liver homogenates from normal and *TTPA*-knockout mice.[4] A single band was present at 32 kD in human and normal mouse liver, but not in the liver of *Ttpa*-knockout mice (data not shown).

Immunohistochemistry

After a 24 h rinse with tap water the fixed samples were dehydrated by graded ethanol and toluene, and then embedded in Histoplast® using the fully automatic Hypercenter®XP. Microtome (MICROM, Walldorf, Germany) sections of 2 μm were mounted on adhesive Superfrost® microscope slides. After microtome sections were dewaxed in toluene and rehydrated, antigen demasking was performed by heat treatment (microwave, 8 min, 850 W). After a 30-min cool down, endogenous peroxidases were deactivated with 3% hydrogen peroxide for 10 min. Unspecific binding sites were blocked with DAKO antibody diluent with background reducing components for 30 min. A 1:5,000 dilution of anti-TTPA rabbit serum in antibody diluent was applied overnight at 4°C in a humid chamber. Specific antibody binding was visualized by biotin-conjugated donkey anti-rabbit IgG (DIANOVA, Hamburg, Germany), 1:800 in antibody diluent, 30 min at 37°C, followed by a streptavidin–biotin–horseradish peroxidase complex (StreptAB-complex/POD) for 30 min, and diaminobenzidine as substrate according to the manufacturer's specifications. Thorough rinsing with phosphate buffered saline, 0.2% Tween 20, was performed after each step. The sections were dehydrated in graded ethanol and toluene and mounted in Entellan®. To test the specificity of the positive signals, control samples were processed the same way with the rabbit pre-immune serum instead of the antibody serum (data not shown). Additionally, the specific signals could be blocked after pre-incubation of the rabbit serum with recombinant TTPA-protein in a final concentration of 50 μg/ml for 30 min at room temperature (Fig. 3H,I).

Co-localization studies were performed by a doublestain with antibodies against human TTPA (rabbit, 1:3,000) and human cytokeratin 7 (mouse, 1:100), a specific marker for trophoblastic cells. Secondary antibodies (biotin-conjugated goat anti-rabbit IgG, 1:800; FITC-conjugated goat anti-mouse IgG, 1:50) were applied at the same time. Biotin was detected by Cy3 conjugated streptavidin (1:150 in PBS). Nuclei were counter-stained with Hoechst 33258. The sections were mounted in Hydromount®. Controls were performed by single immunostaining and substitution of each conjugate with antibody diluent. Microscopic investigations and photographic documentation were performed with a combined light and fluorescence microscope ECLIPSE E-1000 (Nikon, Düsseldorf, Germany), the video camera CCD-1300CB (Vosskühler, Osnabrück, Germany) and the analysis system LUCIA G (Nikon).

RESULTS

Quantification of TTPA-mRNA Transcripts in Various Human Tissues

We developed a TaqMan®-RT-PCR system to specifically quantify *TTPA*-mRNA transcripts in small tissue samples. As a reference system we used the "house-keeping" gene *HPRT* which is part of the purine salvage pathway and thus ubiquitously expressed. The system was designed to exclusively amplify cDNA and not genomic DNA. With cDNA as a template a single band could be seen (data not shown). The calibration curve (Fig. 2A,B) for the serial dilutions from 10^7 to 10^0 copies/μl of cloned *TTPA*- and *HPRT*-cDNA showed a linear regression between the logarithm of copy numbers and of the Ct values. As depicted in Fig. 1B we found in the liver a high *TTPA*/*HPRT*-ratio of 119.4 ± 19.77 (mean ± SD). The *TTPA*/*HPRT*-ratio in placenta was with 2.9 ± 0.95 only 1/40 that of liver. However, it was by a factor of 7–40 above the ratios for fibroblasts (0.406 ± 0.176), lymphoblastoid cells (0.068 ± 0.057) and muscle tissue (0.112 ± 0.048).

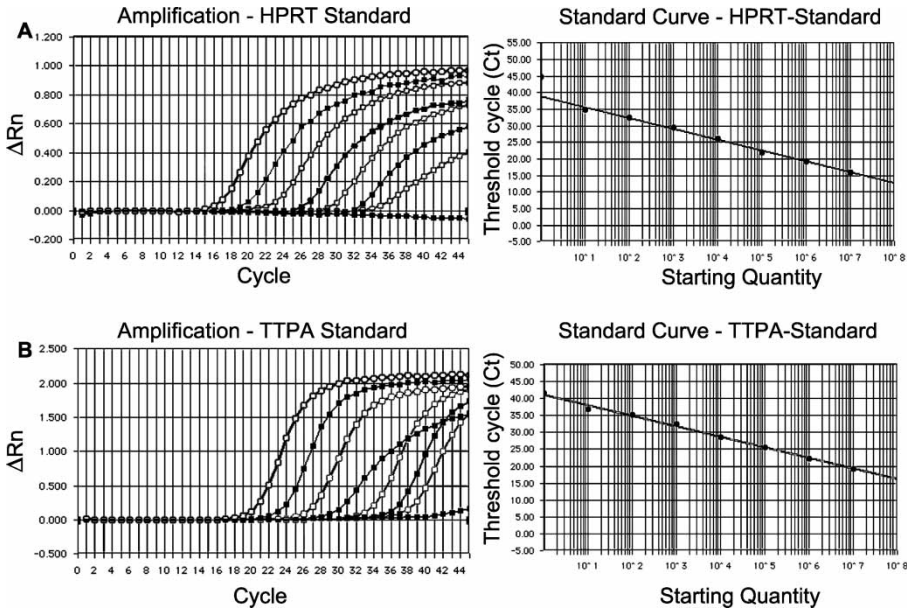

FIGURE 2 Preparation of the calibrator of quantitative PCR for (A) *HPRT* and (B) *TTPA*. Left panel: Increase of fluorescence intensity during cycling. The curves from left to right represent the starting quantity of templates from 10^7 to 10^0 in 1 μl sample. At low dilutions with 1 copy/μl a certain "quantum effect" becomes apparent, since there is a statistical probability that the 1 μl sample used in the reaction contains zero (as in A) or one (as in B) copies of the template. Right panel: Linear regression (HPRT, $R^2 = 0.997$; TTPA, $R^2 = 0.994$) between the logarithms of the starting quantity of cDNA templates and the threshold cycle (Ct).

Measurement of α-Tocopherol Concentrations in the Placenta

We measured α-tocopherol concentrations in the villous tissue of the placenta (Table I). The concentrations of 105 ± 55.2 nmol/g protein (mean ± SD) were below the concentrations present in isolated chorioamnion.[16] There was no correlation ($R^2 = 0.011$) between the *TTPA/HPRT*-ratios and the α-tocopherol content in the trophoblasts of the five placentae.

Detection of TTPA in the Human Placenta

Immunohistochemistry and immunofluorescence with an antibody directed against the N-terminal part of TTPA revealed the presence of TTPA-immunoreactivity in the trophoblast, in the nuclei of the fetal capillaries' endothelium, and throughout the amniotic epithelium (Fig. 3A–G). No signal was found in maternal decidual cells. The TTPA-positive cells in the basal plate could be identified as invasive extravillous trophoblast by specific staining with cytokeratin 7 (Fig. 3F,G). In the amniotic cells, nuclei were markedly stained. All immunohistochemical experiments repeated with the antiserum against the C-terminal TTPA-peptide yielded similar results (data not shown). The specificity of both TTPA-antibodies was demonstrated by immunohistochemical staining of liver tissue with both antisera without and in the presence of rising concentrations of recombinant TTPA-antigen. TTPA-antigen was capable to block the anti-TTPA-staining in liver tissue (Fig. 3H,I). Incubation of liver tissue sections with the respective pre-immune-sera did not yield any signal (data not shown).

DISCUSSION

At first TTPA was assumed to be exclusively expressed in human liver. It was then found in

TABLE I α-Tocopherol concentrations and *TTPA*-copy numbers in the villous portion of the five placentae used in this study. The measurements have been performed in duplicate or triplicate and the values of the single estimations are indicated between slashes. The bottom line depicts the α-tocopherol tissue concentration cited from literature, which investigated isolated chorioamniotic membranes

Tissue	α-Tocopherol concentration [nmol/g protein]	*TTPA*-mRNA copies/*HPRT*-mRNA copies
Villous tissue (placenta #1)	158/152/149	2.910
Villous tissue (placenta #2)	93/106/97	3.335
Villous tissue (placenta #3)	34/33	1.705
Villous tissue (placenta #4)	176/127/200	2.624
Villous tissue (placenta #5)	72/74/77	4.309
Chorioamnion ($n = 10$)	482 ± 232.8 (mean ± SD)[16]	

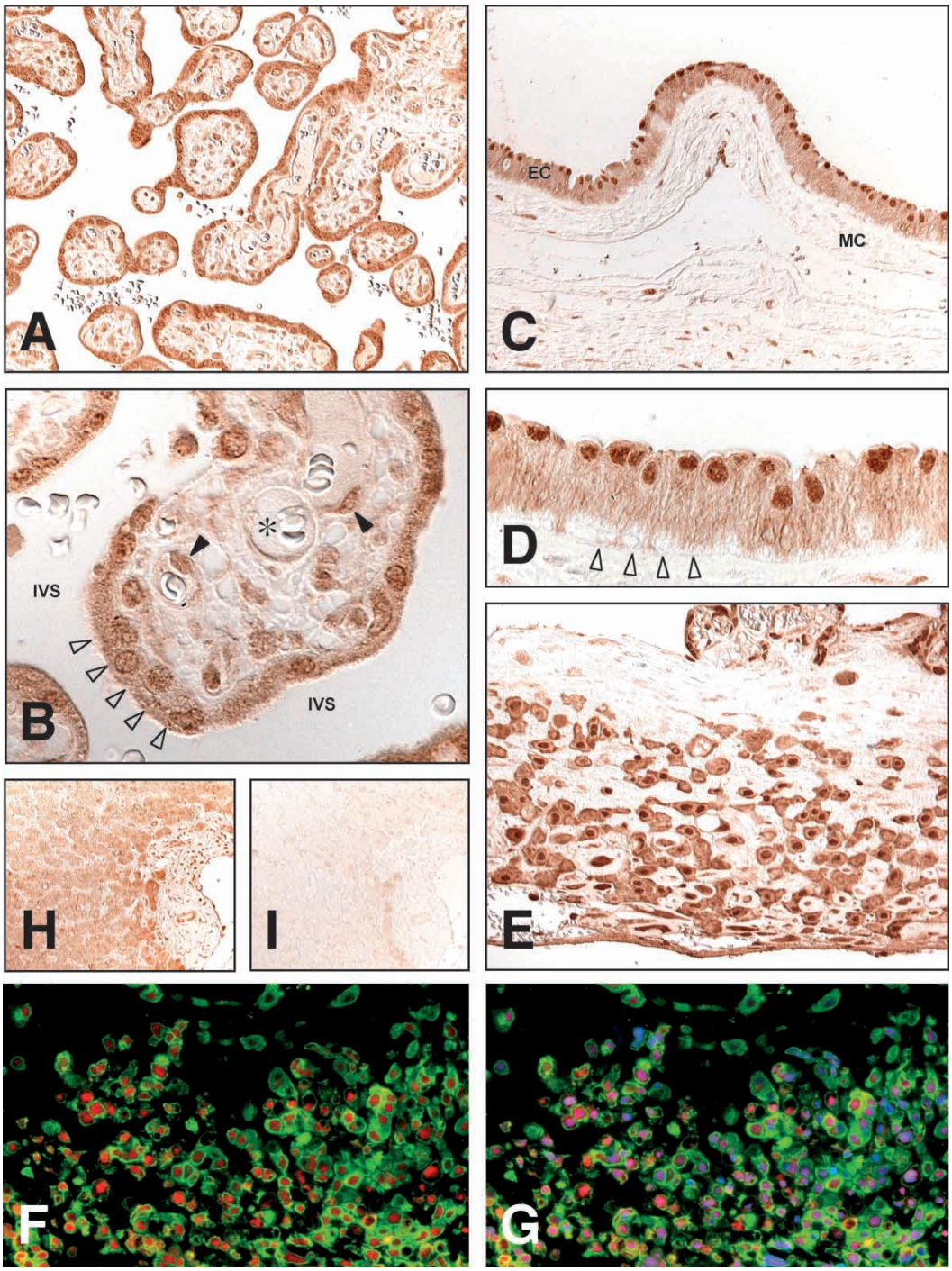

FIGURE 3 Immunohistochemical and immunofluorescent staining of various portions of the human placenta and liver. All sections are labeled with rabbit antiserum prepared against the N-terminal TTPA-peptide. TTPA-positive cells appear brownish due to the diaminobenzidine treatment. (A) Cross-section through the villous part of the placenta (original × 200). (B) Magnification (original × 600) of the central part of plate A, open arrowheads: syncytiotrophoblast. Some isolated TTPA-positive cytotrophoblastic cells can also be seen below the syncytiotrophoblast; closed arrowheads: endothelial cells of the fetal capillaries; star: fetal capillary filled with fetal erythrocytes; IVS: intervillous space filled with maternal erythrocytes. (C) Cross-section through the chorionic plate with the single layered amniotic epithelial cells (EC), which cover the mesenchymal cells (MC) (original × 200). (D) Magnification (original × 600) of the amniotic epithelium. The staining of the nuclei at the luminal side of the epithelium is dominant. Open arrowheads: basal lamina of the amniotic epithelium. (E) Cross-section through the basal plate of the placenta (original × 200) with numerous TTPA-positive cells with predominant nuclear staining. (F) Cross-section through the basal plate of the placenta (original × 200). Superposition of the images of anti-cytokeratin 7 (green) and anti-TTPA (red) shows that TTPA immunoreactvity co-localizes with the trophoblastic maker protein cytokeratin 7. (G) The violet hue of the nuclei demonstrates the nuclear predominance of TTPA immunoreactivity by additional DNA-staining with Hoechst 33258. (H) Staining of liver tissue with the rabbit TTPA-antiserum at a 1:5,000 dilution (original × 200). (I) The same section as plate F stained with TTPA-antiserum at a 1:5,000 dilution after preincubation with TTPA-protein (0.05 μg/μl), which demonstrates the specificity of the antiserum.

the dorsal columns of the spinal cord and in the cerebellum.[17,18] While preparing this manuscript Kaempf-Rotzoll *et al.*[19] reported on the presence of TTPA in the trophoblastic cells of the human placenta. In this study, we show that TTPA is also expressed in various other compartments of the human placenta at term. TTPA-immunoreactivity was detected only in the fetal portion of the placenta (amnion, trophoblast and fetal capillaries' endothelium). The finding of TTPA in human placenta stands in contrast to the results of anti-TTPA-immunostaining and mRNA-studies of the mouse placenta. There TTPA was reported to be absent at any time during development.[4,5] However, the trichorial labyrinthine mouse-placenta differs considerably from the human monochorial villous placenta. This makes extrapolations from mouse to human placental physiology difficult.[20] In human placenta, TTPA is clearly present at the interface between the maternal and fetal circulation, which is composed of the trophoblast and the fetal capillaries' endothelium. In these cells, TTPA appears to be present in the cytosol as well as in the nuclei. In this location TTPA might be in charge of the stereoselective transfer of maternal VLDL-bound *RRR*-α-tocopherol to the fetal plasma.[11] This role that reminds of its established function in the liver. VLDL from the maternal plasma does not cross the placental barrier directly. It is taken up via a very low-density lipoprotein-receptor (VLDLR) at the syncytiotrophoblast and is re-secreted into the fetal circulation. It thus might be more than a coincidence that the VLDLR-mRNA expression in the chorionic villi exactly parallels that of TTPA.[21] However, there was no correlation between the number of *TTPA*-mRNA transcripts and the α-tocopherol content in the trophoblasts of the five placentae. We assume that TTPA is a transport protein, which facilitates the transport of the hydrophobic α-tocopherol between the maternal and fetal circulation. Therefore, the placenta α-tocopherol content at a certain time-point depends not so much on the efficiency of α-tocopherol transport and the number of TTPA-molecules at the placental interface but mostly on other factors. These are the α-tocopherol concentrations of maternal and fetal serum and the velocity of the maternal and fetal blood stream, which brings to and carries away α-tocopherol from the placenta. These additional factors are not likely to be constant in all five placentae prior to delivery. Actually, this finding affirms the assumption that TTPA in the placenta is a transport- rather than a storage protein.

In the amnion epithelium, however, an analogous transport function of TTPA is unlikely for several reasons: (A) these cells do not constitute an interface between fetal and maternal circulation; (B) amniotic fluid lacks VLDL to deliver α-tocopherol to the fetus[22] and (C) the TTPA-immunoreactivity is concentrated in the nuclei of the amniotic epithelium. Taken together, these findings suggest a function of TTPA in the amnion beyond its role in intercellular α-tocopherol transfer. The nuclear localization of TTPA in the amnion epithelium, demonstrated here for the first time (Fig. 3C,D), is indeed intriguing. Until now TTPA was thought to be a cytosolic mediator of the intercellular α-tocopherol transfer towards VLDL.[6] A related protein, however, the α-tocopherol-associated protein (TAP)[23] displays a significant sequence similarity to TTPA and seems to act as a transcriptional activator.[24] Yamauchi *et al.*[24] demonstrated that TAP in the presence of α-tocopherol was transferred from the cytosol into the nucleus and was able to activate transcription of a reporter gene. We can rule out cross-reactivity of our anti-TTPA serum against TAP, since the peptide used for generation of the N-terminal antibody is not part of the homologous CRAL_TRIO domain.[23] Additionally, Western-blotting revealed a single band at 32 kDa and not at 46 kDa as would be expected for TAP. By analogy, a function of TTPA as a transcriptional regulator in amniotic cells may be taken into consideration.

We hypothesize that the TTPA/α-tocopherol system may be relevant to eicosanoid metabolism in the amnion, which is the main source of intrauterine prostaglandins.[25,26] α-Tocopherol may interfere with prostanoid formation by direct inhibition of phospholipase A2[27] or by modulating cyclooxygenase 2 activity.[28] Clinically, such interrelation of the TTPA/α-tocopherol system and eicosanoid metabolism is underscored by the vitamin E responsiveness of preeclampsia, which is evidently mediated by prostaglandins.[29,30]

In conclusion, the cellular and subcellular localization of TTPA in human placenta in part complies with the established role of TTPA as an α-tocopherol-specific protein to transfer the vitamin across tissue interfaces. Other findings, in particular its nuclear localization in amniotic epithelial cells, may add another novel function of α-tocopherol to those detected recently (reviewed in Ref. [31]). This elucidation might finally disclose the vital role of α-tocopherol for embryonic development.

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