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ABC Transporters in Human Placenta and Their Role in Maternal-Fetal Cholesterol Transfer: ABCA1 Candidate Target

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1. Introduction

Placenta is the highly specialized organ of pregnancy, in association with the fetal membranes and amniotic fluids; placenta supports normal fetal growth and development. Primary function of the placenta in all species is to selective transfer of nutrients and waste products between mother and fetus. It participates in the transfer and metabolism of carbohydrates, amino acids, lipids, water, inorganic ions, minerals and vitamins. Respiratory gases also transfer between mother and fetus through placenta. Another important function of placenta is the endocrine function. Adequate nutrient transfer by placenta between mother and fetus during pregnancy is crucial for proper fetal growth and development. Among all nutrients, cholesterol is important as well for proper fetal growth. Embryo and fetal growth phase is the rapid cellular proliferative stage. In this phase, cells differentiate into various cell types. That's why; cells need all the substrates for their proper growth. As long as cells are proliferating, the important cellular structure such as membrane is also vital for the proliferative cells. One of the principal and key components of cell membrane is cholesterol. Cholesterol maintains membrane fluidity and lipid rich microdomain. Any alteration of cellular cholesterol content may lead to diverse complication in different metabolic processes. Cholesterol is the precursor of bile acids, steroid hormones and metabolic regulators such as oxysterols.

Cholesterol is also essential for both activation and propagation of Hedgehog signaling. Sonic Hedgehog (SHH) is responsible for patterning and development of the central nervous system (Porter et al., 1996; Marti & Bovolenta, 2002; Cooper et al., 2003). There are two routes by which cholesterol is available to the fetus, *de novo* synthesis and exogenous source. It has been believed that cholesterol required by the fetus is synthesized by *de novo* cholesterol synthesis by the fetus itself. The individual lacking *de novo* cholesterol synthesis may develop lethal congenital birth defects (Kelley, 2000; Herman, 2003). Convincing evidence shows that maternal cholesterol is a source of fetal cholesterol (Napoli et al., 1997; McConihay et al., 2001). *In vivo* studies in murine and *in vitro* assays using the choriocarcinoma cell line, BeWo have

demonstrated that cholesterol is transported across the trophoblast cells (Schmid et al., 2003; Yoshida & Wada, 2005). ATP-Binding cassette transporter A1 (ABCA1) and ABCG1 are two important transporter involved in cellular cholesterol homeostasis. Recent studies have demonstrated the expression and localization of ABCA1 and ABCG1 in human placenta (Bhattacharjee et al., 2010; Stefulj et al., 2009). Functional involvement of these two transporters in maternal and fetal cholesterol transfer has not been elucidated till now. In this chapter, we will discuss the present knowledge of ABC transporters in human placenta and role of ATP binding cassette transporter A1 (ABCA1) and G1 (ABCG1) on maternal-fetal cholesterol transfer and metabolism through placenta.

2. Sources of fetal cholesterol

Cholesterol is obligatory for all tissues and cells to maintain normal structure and function. The rapidly growing tissues need a significant amount of cholesterol to maintain their cell membrane cholesterol unit, the structural unit of cell membrane. The embryo and fetus are continuously growing and increasing their mass more rapidly compared with adult tissues. In this growth phase, cholesterol, including all other nutrients are crucial for proper development. Cholesterol is available to the tissues by *de novo* synthesis and from exogenous source (i.e. from circulation/diet). It is available to the circulation mainly from diet. The fetal circulation receives maternal circulating cholesterol through placenta. Considering number of evidences show that fetus gets circulation from maternal cholesterol along with its own *de novo* synthesis (Napoli et al., 1997; McConihay et al., 2001). Fetal development includes two different period of growth one is from fertilization to eight week of gestation (embryo) and another is from ninth week of gestation to birth (fetus). At early development, blastocyst is formed by outer layer of trophoblast cells, which invade the endometrium and inner cell mass, and develops into embryo. During this period, maternal blood and remnants of cells digested from trophoblast invasion, immerse the conceptus is an important source of cholesterol (Woollett LA, 2008). Along with the progression of gestation, maternal blood flows into the lacunae of the uterus and forms uteroplacental circulation. The spiral arteries remain plugged in early gestation thus approximately between fourth and eight week of gestation, only small amount of maternal blood leak into the lacunae or intervillous space of the placenta (Burton et al., 1999; Hustin & Schaaps, 1987). At that period, intervillous space contains uterine gland secretion and tiny amount of maternal blood. The uterine glands secrete nutrients, including lipids, to the intervillous space as well (Burton et al., 2002; Hempstock et al., 2004). Thus intervillous space comprises maternal blood containing cholesterol-carrying lipoproteins, and uterine gland secretions, such as lipids and presumably cholesterol. The nutrients would be taken up by syncytiotrophoblasts, will exit through the basolateral side, and diffuse along the stromal channels to the extracoelomic cavity (Woollett, 2008). In an elegant review, Woollett (2008) has discussed the possible source of fetal cholesterol. The Secondary Yolk Sac (SYC) is floating in extracoelomic cavity, the transport of maternally derived nutrients, possibly from the uterine gland secretions and from the maternal circulation, to the embryo occurs through SYC (Enders & King, 1993; Hopkins et al., 1987; Lanford et al., 1991; Perda et al., 1994; Shi et al., 1985). The need for nutrition increases with the progression of gestation and necessitate a more efficient method of nutrient exchange. Thus, the mode of transport of maternally derived nutrition to the fetus becomes primarily hemotrophic as gestation progresses and the placenta becomes the primary route of transport (Woollett, 2008). The spiral arteries are present from the fourth week of gestation but become

functional from eighth week of gestation. When the spiral arteries start functioning, the maternal blood enters into the intervillous space and bathes the syncytiotrophoblasts of the chorionic villi. Maternal nutrients are taken up by the syncytiotrophoblasts by receptor-mediated as well as receptor-independent processes. Once taken up, nutrients cross cells and pass through or between endothelial cells to enter into the fetal circulation (Woollett, 2008). The maternal blood within the intervillous space exchanges three to four times per minute, thus it is an excellent source of nutrients (including cholesterol) for the developing fetus.

3. Routes of maternal-fetal cholesterol transfer

We already know from different observations that maternal cholesterol is a source of fetal cholesterol (Napoli et al., 1997; McConihay et al., 2001). *In vivo* studies in murine and *in vitro* assays using the choriocarcinoma cell line, BeWo have demonstrated that cholesterol is transported across the trophoblast cells (Schmid et al., 2003; Yoshida Wada, 2005). The uptake and utilization of cholesterol by trophoblast through very low density lipoprotein (VLDL) receptor, low density lipoprotein receptor-related protein (LRP), LDL receptor, and Scavenger Receptor B 1 (SR-B1) have been reported (Wadsack et al., 2003; Wyne & Woollett, 1998). Placenta is composed of different cell types, including trophoblasts, endothelial cells, fibroblasts, as well as blood cells in the intervillous space and fetal vessels. But the actual barrier between maternal and fetal circulation is made up of trophoblast cells. In order to acquire maternal cholesterol by the fetus, cholesterol must be taken through the apical side of the placental trophoblast cells and exit through the basolateral side of the trophoblast layer to enter into the fetal circulation. Experimental evidence suggests that trophoblasts efflux cholesterol from cells like any other polarized cells (Woollett, 2005). So far three different mechanisms for cholesterol efflux have been proposed namely aqueous diffusion and protein independent pathway based on concentration gradient, SR-B1 mediated efflux and ABCA1 mediated efflux (Rothblat et al., 1999; Yancey et al., 2003). Apparently all the processes may occur in placenta through the basolateral membrane of trophoblast layer as placenta possesses SR-B1 (Wadsack et al., 2003) and ABCA1 (Langmann et al., 1999). ABCA1 is one of the efflux transporters highly expressed in human placenta (Langmann et al., 1999). It performs cholesterol and phospholipids efflux to lipid poor Apolipoprotein A-1 (ApoA-1), precursor of high density lipoprotein.

4. ABC transporters

The human genome consists of a total of 49 Adenosine-Triphosphate-Binding Cassette (ABC) genes belonging to seven subfamilies named ABCA, ABCB, ABCC, ABCD, ABCE, ABCF and ABCG. The placenta serves an important role as protective barrier as well as in normal fetal development, where ABC transporter plays significant role. ATP-Binding cassette (ABC) transporters perform role in the distribution of nutrients and exchange of metabolites across the placenta. Until now, a number of ATP Binding cassette transporters have been found in human placenta. These include multidrug resistance gene product 1 (MDR1/ABCB1), also known as P-glycoprotein (P-gp), multidrug resistance associated protein (MRPs), Breast Cancer Resistance Protein (BCRP/ABCG2), the Multidrug Resistance-Associated Proteins (MRPs/ABCC1-6 and 7-11) and efflux transporter ABCA1 and ABCG1 (Bhattacharjee et al., 2010; Young et al., 2003). Recently, along with ABCA1, ABCG1 has also been detected in cholesterol efflux activities in human placenta (Stefulj et al., 2009; Aye et al., 2010).

4.1 ATP Binding cassette transporter G1 (ABCG1)

ABCG1 is the transporter responsible for cellular cholesterol efflux. Unlike ABCA1, ABCG1 promotes efflux of cholesterol and oxysterols to HDL whereas ABCA1 predominantly efflux to ApoA1. ABCG1 is expressed both in fetal capillaries and in the syncytiotrophoblast of the placenta (Stefulj et al., 2009; Aye et al., 2010).

4.2 Breast cancer-resistance protein (BCRP)/ABCG2

Breast cancer resistance protein (BCRP) is an ATP dependent transporter also known as mitoxantrone resistance-associated protein (Allikmets et al., 1998) is highly expressed in the placenta, as well as in the uterus (Langmann et al., 2003) and is localized on the apical surface of the chorionic villi syncytiotrophoblast (Litman et al., 2002). The substrate specificity of BCRP has not been elucidated completely. There is considerable overlap of substrates between BCRP and P-gp (Cooray et al., 2002). These include a variety of anti-cancer agents, organic cations and lipophilic conjugates (Sarkadi et al., 2004). The role of BCRP in placenta is not yet known precisely, but from its structure and localization in the placenta, it is assumed that BCRP functions as protective structure in removing cytotoxic drugs from the fetal tissues.

4.3 P-glycoprotein (P-gp/MDR1/ABCB1)

P-glycoprotein (P-gp) is the first and best characterized ABC transporter to be identified so far (Juliano & Ling, 1976). P-gp is expressed in human placenta from first trimester to term (Tanabe et al., 2001). It is localized on the apical membrane of placental syncytiotrophoblasts where it is involved in the extrusion of drug substances from placenta. P-gp is involved with protection of the fetus from entry of harmful substances to the fetus (Lankas et al., 1998; Smit et al., 1999; Ushigome et al., 2000; Samtani et al., 2004). P-gp has an extremely broad substrate specificity. It transports lipophilic drugs that are neutral or cationic belongs to diverse therapeutic categories including antimicrobials (e.g. rifampin), antivirals (e.g. anti-HIV protease inhibitors), anti-arrhythmic (e.g. verapamil) and anti-neoplastics (e.g. vincristine).

4.4 Multidrug resistance gene products 1 and 3 (MDR1, 3) / (ABCB1, ABCB4)

Multidrug resistance gene products are expressed in placenta both in messenger RNA and protein levels (MacFarland et al., 1994). MDR1/ABCB1 is located at the apical surface of the placental syncytiotrophoblast membrane, where it is responsible for the efflux of substrates from trophoblast into the maternal plasma (Litman et al., 2001; Keppler et al., 1998; Bera et al., 2001). These transporters actively extrude different substrates from the cytoplasm including different drugs. Typical substrates of this transporter include some hydrophobic or slightly charged compounds. The hydrophobic/cationic conjugates and drugs include estradiol glucuronide, glucocorticoids, dexamethasone, verapamil, nifedipine, digoxin, paclitaxel, etoposide, vinblastine, doxorubicin, protease inhibitors, fexofenadine, methadone, phenytoin, cyclosporine A, lovastatin etc.

4.5 Multi-drug-resistance associated proteins (MRPs)

Multidrug resistance associated proteins (MRPs) are another family of ATP dependent efflux transporter. There are eight known MRPs so far, six of which have been fully

sequenced (Borst et al., 2000). Their size and function vary greatly among the transporters. It is primarily speculated that MRPs appear to efflux polar compounds and conjugated metabolites in particular unconjugated bilirubin and bile acids (Borst et al., 2000; Bodó et al., 2003). Although there are some controversies about the cellular localization of different MRPs in human placenta, most of the MRPs (MRP1-6, and MRP7) have been found in human placenta (Langmann et al., 2003; Litman et al., 2001; Keppler et al., 1998; Bera et al., 2001; Ozvegy et al., 2001; Sato et al., 2003; Pascolo et al., 2003). MRPs proteins are associated with the removal of glutathione (GSH), glucuronide or sulphate conjugated metabolites from cells (Mathias et al., 2005; St-Pierre et al., 2002). It has also been reported that some unmodified toxins and drugs are transported by the MRP family (Keppler et al., 1998; Nagashige et al., 2003). The specific roles of MRPs in placenta have not been defined, but they perform efflux of polar conjugates xenobiotics or metabolites of endogenous compounds (Pascolo et al., 2003; Leazer & Klaassen, 2003).

4.6 ABCA1

ABCA1 is a 2,261-amino-acid integral membrane protein that comprises two halves of similar structure (Fitzgerald et al., 2001). Each half has a transmembrane domain containing six helices and a nucleotide binding domain (NBD) with two conserved peptide motifs known as Walker A and Walker B, which are present in many proteins that utilize ATP, and a Walker C signature unique to ABC transporters (Dean et al., 2001). ABCA1 is predicted to have an NH₂ terminus oriented into the cytosol and two large extracellular loops that are highly glycosylated and linked by one or more cysteine bonds (Dean et al., 2001; Bungert et al., 2001).

4.6.1 ABCA1 in cellular cholesterol efflux and reverse cholesterol transport

Cholesterol is the integral part of eukaryotic membranes and the precursor of bile acids and of all the steroid hormones. In humans, approximately two thirds of cholesterol is transported by low-density lipoproteins (LDLs) and ~20% by high-density lipoproteins (HDLs); the remaining cholesterol is carried by very low density lipoprotein (VLDL) particles (Attie, 2007). The risk of premature cardiovascular disease is positively correlated with LDL levels and negatively correlated with HDL levels (Attie, 2007). HDL functions as cholesterol acceptor and promotes cholesterol efflux from cells. The ability of HDL to deliver cholesterol to the liver, where it can be secreted into bile and then excreted in the feces, completes a pathway that has been termed 'reverse cholesterol transport' (Attie, 2007). Although ABCA1 was identified as having role in macrophage engulfment of apoptotic cells, the special role of ABCA1 in cholesterol efflux was evident in the year 1999. At 1999, several authors published articles that identified ABCA1 gene mutation in two HDL- deficiency syndromes: familial hypoalphalipoproteinemia and Tangier disease (Rust et al., 1999; Lawn et al., 1999). Since, ABCA1's cholesterol efflux is Apolipoprotein A1 (ApoA1) dependent, ABCA1/ApoA1 association is very important for proper cholesterol efflux. The association of ABCA1 and ApoA1 is not clear enough. Some studies suggest that apoA-1 binds to ABCA1 directly (Oram et al., 2000; Wang et al., 2000; Fitzgerald et al., 2002), whereas some suggest ApoA-1 binding to the cell surface results in a relatively static complex (Smith et al., 2002). An alternative mechanism recently proposed by Chambenoit et al. (2001) saying ApoA-1 initially binds to a lipid domain formed by ABCA1 activity, the "tethered" apoA-1 can then diffuse within the plane of the membrane until contacting ABCA1 (Chambenoit et al. 2001). Regardless of the

nature of its interaction with the cell, apoA-1 has been shown to selectively remove cholesterol that would otherwise be used by Acyl-CoA:cholesterol acyltransferase (ACAT) for esterification/storage (Li et al., 1997). It would stand to reason that an important function of ABCA1 is to promote removal of excess cellular cholesterol which would otherwise be esterified and potentially lead to “foam cell” formation in macrophages. Therefore, two different models have been proposed for the process of apolipoprotein lipidation by ABCA1. The first model suggests that apolipoproteins interact with ABCA1 at the cell surface and excess cellular cholesterol is transported to the plasma membrane by a vesicular mechanism, possibly packaged in the Golgi (Oram, 2003). The second model is based on evidence suggesting that ApoA1 and ABCA1 interact at the cell surface and then internalized where lipidation actually takes place in the lumen of an endosome from which the cholesterol is taken (Takahashi and Smith, 1999; Santamarina-Fojo et al., 2001). ABCA1 has been shown to recycle between the plasma membrane and the endosomal/lysosomal compartments (Neufeld et al., 2001). However, this recycling may be involved in the regulation of ABCA1 degradation rather than its function (Oram, 2003). It should also be considered that both models are correct and contribute in some way to pre- β HDL particle formation. There is some evidence that ABCA1 is actually a phospholipid “pump” or “floppase” instead of moving cholesterol itself. One study was able to pharmacologically uncouple phospholipid from cholesterol removal (Fielding et al., 2000). This finding has led to a hypothesis that ABCA1 lipidates ApoA1 with phospholipid only and the newly formed pre- β HDL obtains cholesterol by diffusional means from cholesterol-rich lipid rafts or caveolae in a “two-step” mechanism (Fielding et al., 2000; Wang et al., 2001). Others have found that ABCA1 does not use caveolae or lipid raft cholesterol (Mendez et al., 2001). Interestingly, one group did report that ABCA1 promotes cholesterol removal from “lubrol rafts” (defined by the authors as lipid rafts soluble in Triton-X, but insoluble in the detergent lubrol) (Drobnik et al., 2002). ABCA1 activity has also been shown to increase outer plasma membrane leaflet phosphatidylserine(PS) exposure-leading to speculation that PS may play a role in ApoA1 binding and lipidation (Hamon et al., 2000; Chambenoit et al. 2001). This result was more closely examined and PS was found not to mediate apoA1 binding or cholesterol removal (Smith, 2002). The increase in PS exposure is likely a side effect of prolonged, increased ABCA1 expression which can damage cell membranes (Oram, 2003). Although ABCA1 may function as a phospholipid translocase, it is feasible that cholesterol is still able to be moved with the phospholipid (Oram, 2002). ABCA1 mediated transport of other molecules have been discovered including α -tocopherol (Oram et al., 2001), ApoE (Von Eckardstein et al., 2001), and interleukin-1 β (Zhou et al., 2002). P-glycoprotein, a close relative of ABCA1, is predicted to have a central pore or pocket of 3 nm in diameter (Higgins et al., 1997). An opening of this size would allow many molecules to be transported together. Therefore, considering the variety of molecules transported by ABCA1, it can be envisioned that ABCA1 functions by transporting lipophilic molecules complexes with phospholipid including cholesterol (Oram, 2002). Indeed, using a transfected cell line with an inducible ABCA1 promoter revealed that a “two-step” cholesterol removal mechanism is unlikely (Vaughan & Oram, 2003).

4.6.2 ABCA1 in cellular apoptosis and proliferation

Hamon et al (2000) provided evidence that several cellular functions are controlled by ABC1 (now said ABCA1). Optimal engulfment of cell corpses generated by apoptosis is hampered by lack of ABC1 function and enhanced by it's over expression. Very recently, Yvan-Charvet

et al (2010) showed ABCA1, ABCG1, and HDL inhibit the proliferation of hematopoietic stem and multipotential progenitor cells (HSPCs). They suggested the proliferation of HSPCs is regulated by cholesterol efflux mechanisms involving LXRs, ABCA1, ABCG1, and HDL (Yvan-Charvet et al., 2010). In the same article, Yvan-Charvet et al (2010) speculated that increased membrane cholesterol content secondary to ABC transporter deficiency results in increased cell-surface expression of the common β subunit of the IL-3/GM-CSF receptor that, in turn leads to increased downstream Ras/Erk signaling and increased proliferative response to IL-3 and GM-CSF. In mammalian cells, cholesterol, glycolipids, and proteins are organized in lipid rafts in the plasma membrane. ABCA1 and ABCG1 transport excess cholesterol from plasma membrane to form HDL. In HPSCs, growth factor receptors are organized in lipid rafts to promote receptor signaling and consequently, cell proliferation and migration (Giebel et al., 2004). As cholesterol overload can cause havoc in cells, its concentration is regulated by several mechanisms. Excess cholesterol is removed by ATP binding cassette (ABC) transporters in the plasma membrane, which move cholesterol to extracellular HDL particles at the cell surface (Tall, 2008). Recently it was shown in HSPC cells that when cholesterol is removed from this type of cells, the membrane raft disassemble, receptor signaling (such as through the IL-3 receptor) is hampered, and receptor dependent outcomes such as cell proliferation are reduced (Yvan-Charvet et al., 2010; Hansson & Björkholm, 2010).

4.6.3 ABCA1 in inflammation

Until now, we have seen the function of ABCA1 in cellular cholesterol efflux, cellular apoptosis, proliferation and reverse cholesterol transport and as an important target for atherosclerosis treatment (Tall, 2008; Schmitz & Grandl, 2008). Several recent studies showed that ABCA1 is also involved in inflammation and/or immune response. Studies with ABCA1 knockout mice demonstrated the relationship between ABCA1 and inflammation (Yvan-Charvet et al., 2008; Koseki et al., 2007; Schmitz et al., 1999; Aiello et al., 2003; Zhu et al., 2008; Francione et al., 2005; McNeish et al., 2000; Christiansen-Weber et al., 2000). Although the precise mechanism that ABCA1 plays a key role in modulating inflammatory response remains to be elucidated, several studies shown that the cholesterol export activity of ABCA1 could account for its potent anti-inflammatory properties (Koseki et al., 2007; Zhu et al., 2008; Murphy et al., 2008; Tellier et al., 2008). ABCA1 has been also reported in the regulated secretion of macrophage migration inhibitor factor (MIF) (Flieger et al., 2003). MIF is the pleiotropic multifactorial cytokine with a mostly proinflammatory spectrum of action in the host immune response. MIF is a critical mediator of a number of immune and inflammatory conditions (Calandra & Bucala, 1996; Mitchell & Bucala, 2000; Lue et al., 2002).

4.6.4 ABCA1 in placenta

The existence of ABCA1 in human placenta was first identified by Langmann and his team in mRNA levels in the year of 1999. Thereafter, it received more attention when it is known that maternal cholesterol is an important source of fetal cholesterol and Christiansen-Weber et al (2000) showed ABCA1 malfunction resulted in severe placental malformation, structural abnormalities, intrauterine growth restriction and increased neonatal death. Until now there are few publications showing ABCA1 expression and localization in human placenta. We

showed the expression and localization of ABCA1 at both first trimester and term human placenta (Bhattacharjee et al., 2010).

Western blotting and real time PCR analysis showed that ABCA1 is expressed both in first trimester and term placenta and its expression does not differ between first trimester and term placenta both in protein and mRNA levels (Figure 1). We also showed the localization of ABCA1 by immunohistochemistry and immunofluorescent staining both in first trimester and term placental tissues (Figure 2). We found that ABCA1 is more predominantly localized at the basolateral and infrequently at the apical part of the cytotrophoblast cell layer of first trimester human placenta (Bhattacharjee et al., 2010). It was also localized in some other cell types of the placenta including stromal and endothelial cells of chorionic villi. In term placenta, the localization was observed in few villous cytotrophoblast and endothelial cells of the placental vasculature. Although, we found a very punctuate staining in the syncytial layer of the placenta some other researchers showed the expression of ABCA1 in the syncytial layer of placental epithelial layer. Different sampling variation and different antibodies might be responsible for not being exact results. In a nut shell, from the

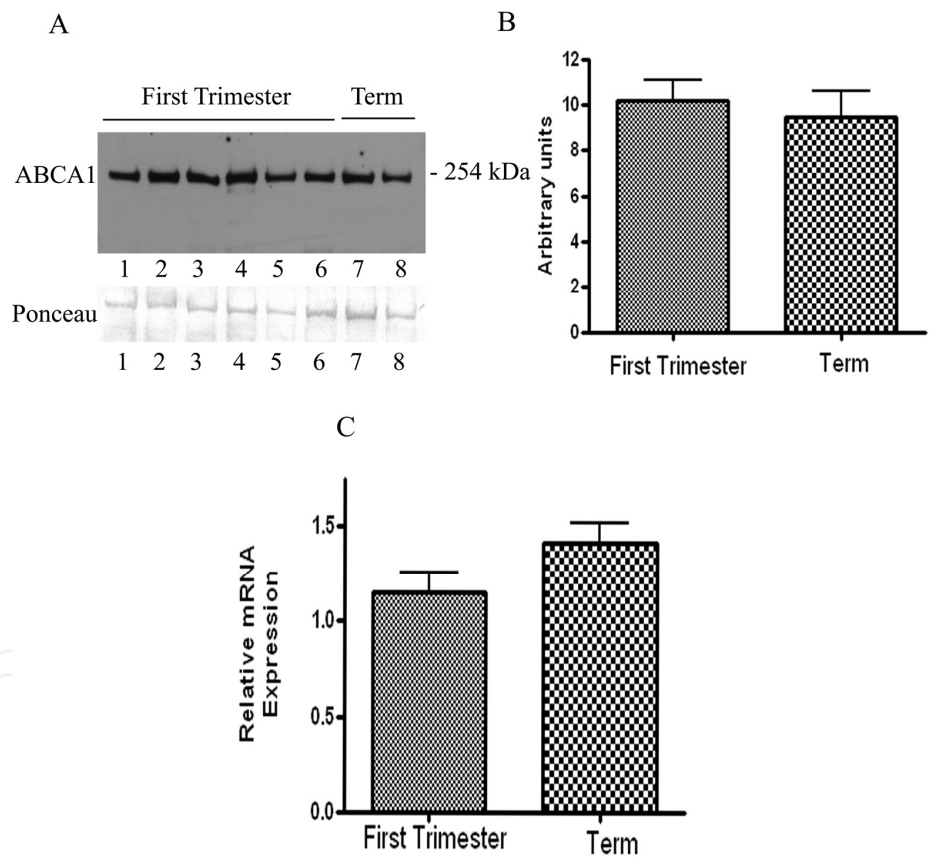


Fig. 1. Western blot and qRT-PCR analysis of ABCA1 in human placenta tissues. (A) Top, representative western blot analysis of ABCA1 protein at first trimester (lanes 1-6) and term human placenta (lanes 7-8) using mouse anti-human ABCA1 monoclonal antibody. Bottom, Membrane stained with ponceau S to assess total loaded protein in each lane. (B) The histogram represents densitometric measurement of western blot bands of first trimester and term placental tissues. (C) Quantification of ABCA1 mRNA in human placenta during the first trimester and term of gestation using qRT-PCR (Reproduced from Bhattacharjee et al., 2010 with the permission from Elsevier).

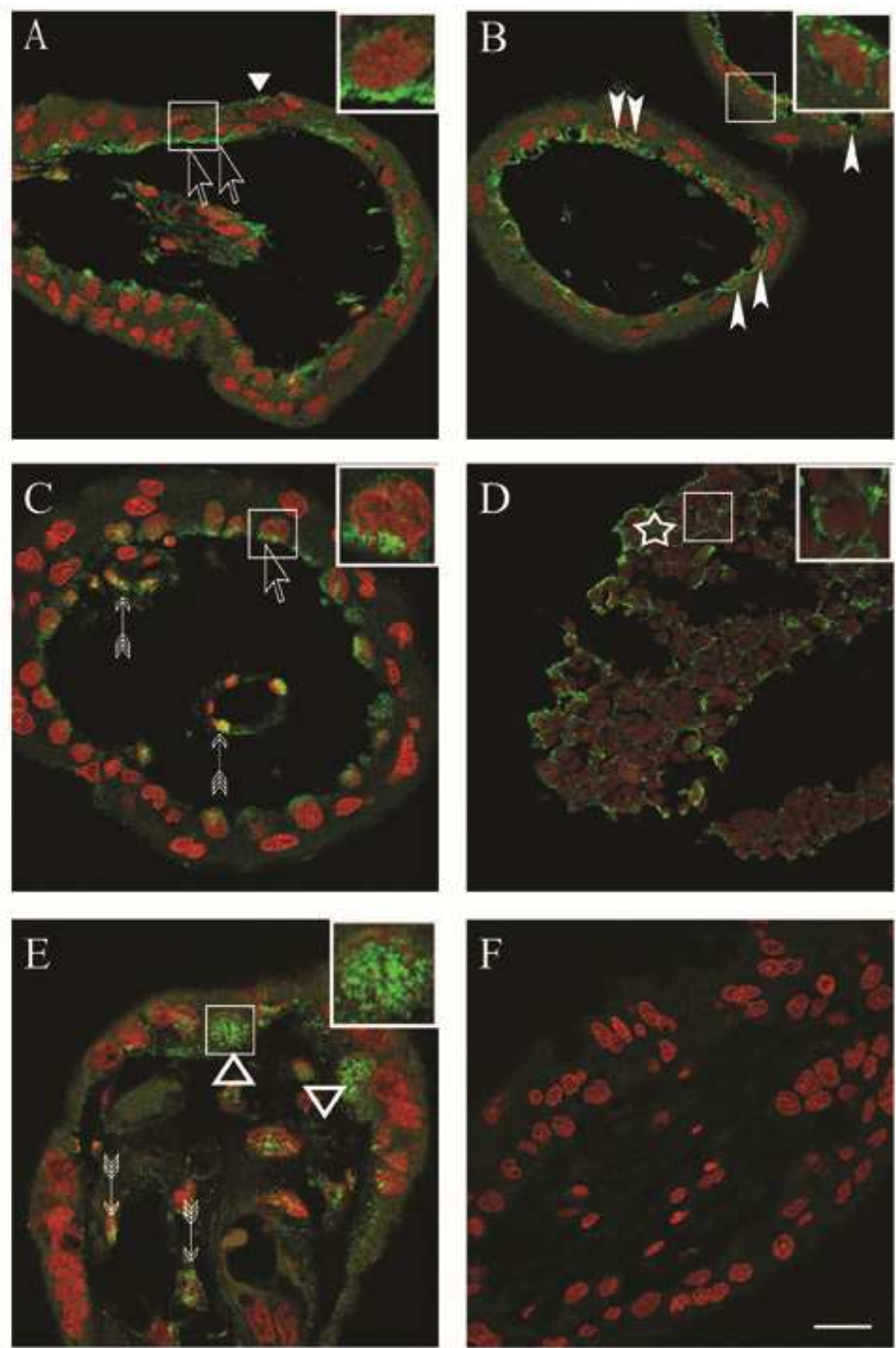


Fig. 2. Confocal laser scanning microscopic localization of ABCA1 using mouse anti-human ABCA1 monoclonal antibody in first trimester (A-D) and term (E) human placenta. Positive staining of ABCA1 is shown in green. Red staining represents nuclei (propidium iodide stained). In first trimester placenta, ABCA1 staining was found in the basolateral (white arrowheads) and in the apical part (black arrow heads) of villous cytotrophoblast cell membrane, rarely observed staining in syncytial brush border (white triangle) and in endothelial cells (black arrows) of the villous core (A-C). (D) ABCA1 positive staining in the cell membrane of most extravillous cytotrophoblast cells (white star frame). In term placenta (E), diffuse ABCA1 positive staining in the cytotrophoblast cells (white triangle frame). (F) Negative control in first trimester placenta. Inserts show the higher magnification of the selected part. Scale bar= 20 mm for all the images (Reproduced from Bhattacharjee et al., 2010 with the permission from Elsevier).

studies till now, it is evident that ABCA1 is expressed both in cytotrophoblast and syncytiotrophoblast layer of the placental along with hofbauer cells and endothelial cells of the placenta. These finding together support the role of ABCA 1 in fetoplacental transport function. The different localization of ABCA1 in different cells types may have different functions, which remains to be elucidated.

In term placental tissues, ABCA1 is present in cytotrophoblast cells but diffuse in the whole cell without any specific localization. Some staining in the term placental cytotrophoblast cells suggests it to be in intracellular endocytic compartment. ABCA1 is actually a membrane transporter although its expression in the intracellular endocytic compartment has also been reported in different cell types (Hamon et al., 2000; Cooper et al., 2003)

4.6.5 Role of ABCA1 in Cholesterol transfer through human placenta

As placenta is the vital organ and a site for nutrient and waste exchange between mother and fetus, placental transport and metabolism of cholesterol and lipids are critical for the fetal development and its survival. Cholesterol is an integral part of cell membranes, precursor of steroid hormones such as progesterone and metabolic mediators such as oxysterol (Woollett, 2005). Cholesterol is essential for both activation and propagation of Hedgehog signaling (SHH), responsible for patterning and development of the central nervous system (Porter et al., 1996; Marti & Bovolenta, 2002; Cooper et al., 2003). There are two routes by which cholesterol is available to the fetus, the *de novo* synthesis and exogenous source. The individual lacking *de novo* cholesterol synthesis may develop lethal congenital birth defects (Kelley, 2000; Herman, 2003).

Dysfunction of ABCA1 in mice resulted in severe placental malformation with structural abnormalities, intrauterine growth retardation and increased neonatal death (Christiansen-Weber et al., 2000). Reduced expression of placental ABCA1 was observed in women with antiphospholipid syndrome (Albrecht et al., 2007) and ABCA1 was reported as a potential target for *in utero* therapy of Smith-Lemli-Opitz syndrome (Lindegaard et al., 2008). Recently, Stefulj et al. (2009) demonstrated the presence of ABCA1 in endothelial cells of term placenta and its involvement in cholesterol transfer towards fetal circulation. However, no information is available on the regulation of ABCA1 expression and its functions in human placenta.

By using florescent tagged cholesterol in first trimester placental explants as an *ex-vivo* model, we found that ABCA1 is significantly involved in cholesterol efflux in human placenta explants (Unpublished observation). Further functional studies are required to explore the role of ABCA1 in maternal fetal cholesterol transfer and metabolism. Recently, there are few studies showed that silencing of ABCA1 and pharmacological inhibition of ABCA1 by glyburide decreased cholesterol efflux to Apolipoprotein A-1 in cultured primary trophoblast cells (ApoA-1) (Aye et al., 2010). On the other hand they also showed that endogenous receptor induction by synthetic LXR α/β inducer have increased ABCA1 and enhanced cholesterol efflux to ApoA-1 (Aye et al., 2010).

Two mechanisms have been suggested to explain ABCA1-mediated cholesterol efflux to apoA-1 (Takahashi & Smith, 1999; Oram et al., 1991; Chen et al., 2001). The apoA-1 forms complexes with phospholipid and cholesterol at the cell surface in a process promoted by ABCA1 activity (Chen et al., 2001). Alternatively, apoA1 binds ABCA1 at the cell surface

and the complex is subsequently internalized and targeted to late endosomes, where apoA1 picks up lipids. The apolipoprotein-ABCA1-lipid complexes are then resecreted from the cell by exocytosis (Chen et al., 2001). Azuma et al. (2009) have shown that apoA-1 internalizes inside the cell and colocalizes with the cell surface-derived ABCA1 on endosomal compartment contributing to HDL formation when excess lipoprotein-derived cholesterol has accumulated in cells.

Thus in light of our results we can speculate that at term of gestation, the pathway involving the internalization of apoA1/ABCA1 complex might prevail on the other one operating possibly at first trimester.

During the first trimester of pregnancy cholesterol is crucially important for both fetal and placenta development (Napoli et al., 1997). Along with *de novo* cholesterol synthesis, fetal plasma cholesterol concentration is significantly correlated with the maternal one and the significance is greatest in the fetuses that are less than 6 months old (Woollett, 2001) suggesting maternal cholesterol supply is crucially important during the earlier phases of development.

Although it is believed that maternal cholesterol level is correlated with fetal cholesterol, still now no direct evidence exists about the maternal cholesterol entrance through the placenta nor cholesterol trafficking and efflux by ABCA1 in human first trimester placenta. By using a chorioncarcinoma cell line Schmid et al (2003) have shown the ability of placental cells to transport maternal derived cholesterol. In the same study the ABCA1 mediated cholesterol efflux was not apparent, and despite multiple manipulations to up-regulate the expression of ABCA1, there was no increase of cholesterol efflux to exogenous apoA1.

Significant involvement of ABCA1 in cholesterol efflux at the maternal fetal interface has been recently demonstrated by several studies on endothelial and cytotrophoblast primary isolated cells from term placental tissues (Stefulj et al., 2009; Aye et al., 2010).

5. Other ABC transporters involved in cholesterol transfer

Many ABC transporters are involved in cholesterol homeostasis by participating transferring lipid molecules including cholesterol. Unlike ABCA1 and ABCG1, ABCG5 and ABCG8 expressed in liver, and function in the secretion of biliary cholesterol. ABCG5 and ABCG8 restrain cholesterol absorption in the lumen of the intestine by excreting absorbed cholesterol. These transporter help in the removal of excess cholesterol from the body and maintain cholesterol homeostasis. ABCG1 is also expressed in the brain along with placenta. ABCG1 and ABCG4 are expressed in the brain, function in cholesterol metabolism in the central nervous system (CNS) (Matsuo, 2010). ABCA2 has also been reported to be involved in cholesterol efflux. ABCA2 is also expressed in animal and human placenta but its role in placenta has not been studied precisely still now (Burke et al., 2009).

6. Conclusion and future explorations

Although cholesterol is the fundamental factor for normal fetal development, there is evidence suggesting that an excess of maternal cholesterol can have both acute and chronic detrimental effect to the fetus health. Maternal hypercholesterolemia is correlated with the fatty streaks aortas in the fetus that persist in childhood (Napoli et al., 1997). This fatty

streak formation has been suggested as a programming mechanism target for the development of atherosclerosis later in life (Palinski et al., 2002). Considering the importance and detrimental efflux of cholesterol, cholesterol transferring pathway including ABC transporter can be a target for *in utero* therapy to control the anomalies derived from cholesterol deficiency or adequacy. In a recent study ABCA1 has been detected as a target for *in utero* therapy of Smith-Lemli-Opitz Syndrome (SLOS), a congenital anomaly due to error of cholesterol synthesis (Lindegaard et al., 2008).

In addition to its lipid export activity, it has been demonstrated that ABCA1 plays an important role in immune responses (Zhu et al., 2008; Tanc et al., 2009; Yvan-Charvet et al., 2010). Mice lacking ABCA1 have an enhanced inflammatory response to lipopolysaccharide (LPS) (McNeish et al., 2000). Incubating apoA-1 with activated ABCA1-expressing macrophages suppressed production of the inflammatory cytokines interleukin 1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) (Tanc et al., 2009). Thus the expression of ABCA1 in the villous and extravillous trophoblast may reflect additional functions other than those involved in cholesterol homeostasis. Our group has recently been demonstrated that 17 beta estradiol modulate placental MIF secretion by regulating the expression of ABCA1 transporter protein (Ietta et al., 2010).

Although ABCA1 and ABCG1 are involved in placental cholesterol efflux, this is more likely to occur by the involvement of other mechanisms along with simple diffusion such as via SR-B1, fatty acid-binding proteins (FABPs) and other transporters. Further studies on these transporters will clarify the complete placental cholesterol transfer mechanisms.

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This book contains the total of 19 chapters, each of which is written by one or several experts in the corresponding field. The objective of this book is to provide a comprehensive and most updated overview of the human placenta, including current advances and future directions in the early detection, recognition, and management of placental abnormalities as well as the most common placental structure and functions, abnormalities, toxicology, infections, and pathologies. It also includes a highly controversial topic, therapeutic applications of the human placenta. A collection of articles presented by active investigators provides a clear update in the area of placental research for medical students, nurse practitioners, practicing clinicians, and biomedical researchers in the fields of obstetrics, pediatrics, family practice, genetics, and others who may be interested in human placentas.

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