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LIIS UUSKÜLA

Placental gene expression in normal and complicated pregnancy

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Placental gene expression in normal and complicated pregnancy

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred to in the thesis by Roman numerals:

I Nagirnaja L, Rull K, **Uusküla L**, Hallast P, Grigorova M, Laan M. Genomics and genetics of gonadotropin beta-subunit genes: Unique *FSHB* and duplicated *LHB*/*CGB* loci. Mol Cell Endocrinol. 2010 Nov 25; 329 (1–2):4–16.

II Rull K, Hallast P, **Uusküla L**, Jackson J, Punab M, Salumets A, Campbell RK, Laan M. Fine-scale quantification of HCG beta gene transcription in human trophoblastic and non-malignant non-trophoblastic tissues. Mol Hum Reprod. 2008 Jan 14(1):23–31.

III **Uusküla L**, Rull K, Nagirnaja L, Laan M. Methylation allelic polymorphism (MAP) in chorionic gonadotropin beta5 (CGB5) and its association with pregnancy success. J Clin Endocrinol Metab. 2011 Jan 96(1):E199–207.

IV **Uusküla L**, Männik J, Rull K, Minajeva A, Kõks S, Vaas P, Teesalu P, Reimand J, Laan M. Mid-gestational gene expression profile in placenta and link to pregnancy complications. PLoS One. 2012 Nov 7(11):e49248.

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My contributions to these articles are the following:

Ref. I – contributed to the preparation of the review article

Ref. II – participated in experimental conduct and in manuscript preparation

Ref. III – contributed as the leading researcher, participated in experimental design, performed the experiments and data analysis, leaded data interpretation and wrote the first draft of the paper

Ref. IV – contributed as the leading researcher, participated in experimental design, carried out most of the experiments, data analysis and interpretation, wrote the first draft of the paper

LIST OF ABBREVIATIONS

AGA	appropriate for gestational age
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BMP5	*bone morphogenetic protein 5 gene*
C14MC	chromosome 14 microRNA cluster
C19MC	chromosome 19 microRNA cluster
CCNG2	cyclin-G2 protein (*CCNG2* for *gene*)
CDH11	*cadherin-11 gene*
CGB	*chorionic gonadotropin β-coding gene*
CSH	chorionic somatomammotropin hormone (*CSH* for *gene*)
EP	ectopic pregnancy
ETP	elective termination of pregnancy
FDR	false discovery rate
FGR	fetal growth restriction
FSH	follicle stimulating hormone
FST	*follistatin gene*
GAPDH	*glyceraldehyde phosphate dehydrogenase gene*
GATM	*glycine amidino-transferase gene*
GD	gestational diabetes
GH	growth hormone (*GH* for *gene*)
GO	gene ontology
GPR183	*G-protein coupled receptor 183 gene*
HCG	human chorionic gonadotropin
HCGβ	*human chorionic gonadotropin β-coding gene*
HPRT1	*hypoxanthine phosphoribosyltransferase 1 gene*
IUGR	intra-uterine growth restriction
ITGBL1	*integrin beta-like 1 gene*
kb	kilobase
LGA	large for gestational age
LH	luteinizing hormone
LHB	*luteinizing hormone β-coding gene*
lncRNA	long non-coding RNA
LYPD6	LY6/PLAUR domain containing 6 protein (*LYPD6* for *gene*)
MAP	methylation allelic polymorphism
MEG3	*maternally expressed gene 3*
mole	molar pregnancy
mRNA	messenger ribonucleic acid
NEDD9	*neural precursor cell expressed developmentally down-regulated 9 gene*
NR3C1	*nuclear receptor subfamily 3 group C member 1 gene*
NRCAM	*neuronal cell adhesion molecule gene*
PE	preeclampsia

PL	placental lactogen
PLAGL1	*pleiomorphic adenoma gene-like 1 gene*
RM	recurrent miscarriage
RT-qPCR	real time quantitative polymerase chain reaction
SGA	small for gestational age
SLC16A10	*solute carrier family 16 member 10 gene*
SNP	single nucleotide polymorphism
STC1	stanniocalcin-1 protein (*STC1* for *gene*)
ZFP36L1	*zinc finger protein 36 C3H type-like 1 gene*
TSH	thyroid stimulating hormone
UTR	untranslated region

1. INTRODUCTION

Placenta is a unique temporary feto-maternal endocrine organ that mediates all interactions between mother and fetus over the period of gestation. Successful outcome of pregnancy requires dynamic regulation of molecular, histological and functional changes in placenta to guarantee normal fetal growth and development as well as maternal adaptation to pregnancy. Besides its leading role in shaping *in utero* environment, placenta also influences the health of mother and child in later life.

Due to rapid species-specific evolution, placenta is structurally one of the most variable organs among mammals. Rapid evolution is also characteristic to placenta-specific genes. Human placenta has a unique transcriptional landscape that involves active retrotransposon genes, primate-specific families of duplicated genes, novel microRNA clusters, and high incidence of epigenetic regulation through mechanisms such as DNA methylation and genetic imprinting. Temporal and spatial regulation of gene expression in placenta is crucial for its endocrine function. Importantly, altered placental gene expression is associated with pregnancy complications.

The human chorionic gonadotropin (HCG) is a glycoprotein and a crucial pregnancy hormone synthesised by the placental syncytiotrophoblast. HCG facilitates the implantation and hemochorial placentation in primates and is responsible for maintaining early pregnancy. HCG is composed of an α-subunit shared with other gonadotropic glycoproteins, and a specific β-subunit encoded by a set of primate-specific duplicated *HCGβ-coding* (*CGB*) genes with nearly 99% sequence identity (*CGB8*, *CGB5*, *CGB*, *CGB7*). Although HCG hormone levels are subject to great inter-individual variability, its hormone expression in serum is clearly informative of pregnancy complications. Low level of HCG in maternal serum during the first trimester of pregnancy is related to miscarriage and extrauterine pregnancy, while increased HCG expression may refer to molar pregnancy or trisomy 21.

As *in utero* development has a great role in the well-being of mother and child during pregnancy and later in life, understanding the role of placenta in healthy and complicated pregnancy is of great clinical and societal importance. However, placenta remains under-investigated in current biomedical research, often due to complex and ethically restricted sample collection procedures. The global patterns of spatial and temporal gene expression throughout the entire pregnancy have been poorly characterised to date. In particular, little is known about the gene regulation of human mid-gestational placenta. More work is required to understand the early transcriptional and epigenetic mechanisms that regulate the *HCGβ* genes and ultimately determine the success of pregnancy. In addition, the altered placental gene regulation in complicated pregnancies requires deeper exploration. Early pregnancy complications, such as miscarriage and ectopic pregnancy, are associated with altered implantation, placentation, angiogenesis and immune tolerance. Term pregnancy complications (pree-

clampsia, gestational diabetes mellitus, fetal growth anomalies) are related to metabolic dysfunctions. Investigation of these topics will help characterise molecular mechanisms of human pregnancy, lead to a greater understanding of pregnancy complications, and reveal biomarkers that will help predict pregnancy outcome.

In this doctoral thesis, I investigated three aspects of placental gene regulation in healthy and complicated pregnancies. First, I showed that decreased level of *HCGβ* expression is potentially guided by DNA methylation, leading to decreased hormone HCG level and complications in early pregnancy. Second, I performed the first dynamic analysis of global gene expression in human placenta of early and mid-gestation pregnancies. Third, I detected aberrant expression of mid-gestation marker genes in term pregnancy complications. This work revealed potential novel biomarkers for early detection of maternal and fetal complications, including a promising maternal blood plasma marker Stanniocalcin-1 (STC1) that may be developed into a non-invasive biomarker to diagnose cases of preeclampsia and fetal growth restriction.

2. LITERATURE OVERVIEW

2.1. Placenta – a temporary, rapidly evolving organ in mammalian gestation

Placenta is a unique and temporary organ that binds two genetically distinct individuals, the fetus and the mother, and mediates their interactions over gestation. Dynamic regulation of molecular, histological and functional changes in placenta guarantees fetal development and the adaptation of maternal metabolism during pregnancy. Placenta is genetically identical to the fetus and it thus provides valuable material for studies of developmental biology, human genetics and biomedicine.

2.1.1. Structure of human placenta

Human placenta is a chimerical feto-maternal organ composed of the fetal part (also known as chorion) and the maternal part (decidua). Placental development is initiated at the implantation of the embryo when the outer layer of blastocyst cells starts to invade the maternal endometrium (**Figure 1**). The developing fetus originates from embryoblast cells (**Figure 1**) (Kaufmann and Baergen, 2006).

Human placentation begins with implantation when the blastocyst becomes completely embedded within the maternal decidualized endometrium (**Figure 1**) (Jauniaux *et al.*, 2006). The trophoblast forms a double-layer membrane composed of syncytiotrophoblast and cytotrophoblast cells (**Figure 2B**). The outer multinuclear syncytiotrophoblast layer is formed in the fusion of numerous trophoblastic cells and it remains in direct contact with maternal blood for most of the pregnancy (Kliman, 2000). The inner layer of cytotrophoblast represents the proliferative stem cells of trophoblast (Kaufmann and Baergen, 2006). Rapid proliferation of trophoblast cells leads to branching of chorionic villi and further destructive invasion into maternal tissues (Boyd, 1970; Castellucci *et al*., 2000). Placental development during the first trimester of pregnancy occurs under low oxygen conditions and limited uterine blood flow, as the invading trophoblast cells plug the ends of maternal spiral arteries (Burton *et al*., 2010; Rodesch *et al*., 1992). First trimester growth of human placenta and fetal development during normal pregnancy is remarkably similar among individuals. However, considerable inter-individual variation occurs in fetal and placental growth during the second and third trimesters. This variability is likely caused by the fact that maternal blood starts to flow into intervillous space only by the 12^{th} week of pregnancy (Jauniaux et al., 2006). The growth variation characteristic to later gestation is associated with the differential remodelling of spiral arteries in the first trimester. Late pregnancy complications such as preeclampsia and fetal growth restriction may be caused by altered placentation; for

example, aberrant programming of placental development during early gestation, abnormal remodelling of spiral arteries, and decreased invasion of extravillous trophoblast.

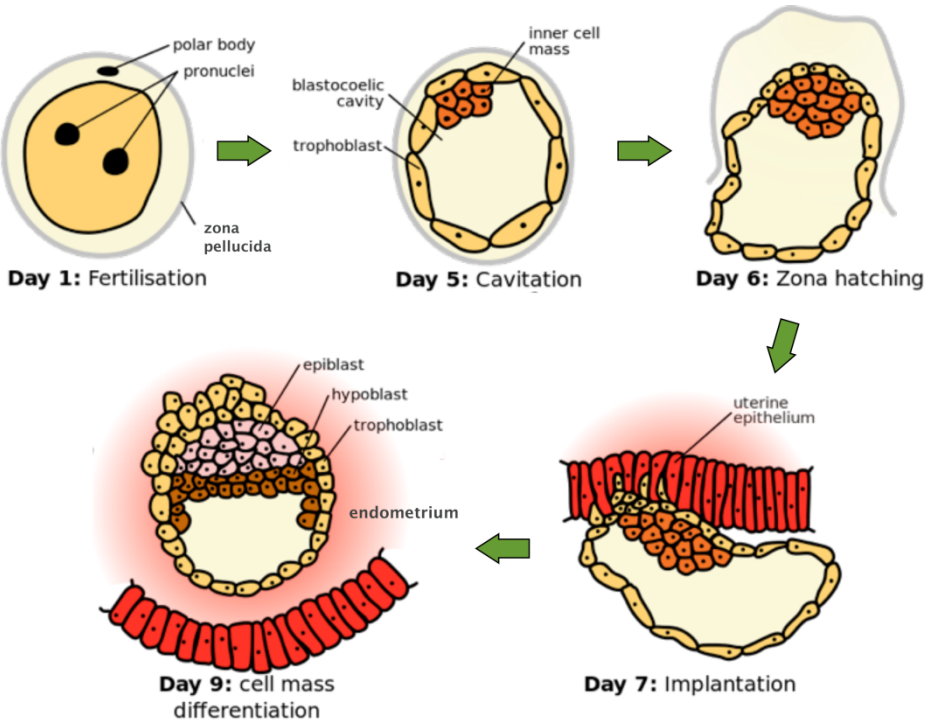

Figure 1. Schematic representation of blastocyst implantation (modified from Wikipedia).

The main functional units of mature human placenta are the fetally derived placental villi arranged in tree-like structures (**Figure 2A, B**). The villi are closely packed in intervillous space that is highly vascularised and filled with maternal blood in order to establish maternal-fetal gas and nutrient exchange (**Figure 2A, B**). Fetal circulation enters the placenta through the umbilical cord arteries, whereas nutrients and oxygen are transported to the fetus via umbilical cord vein (Blackburn, 2003). The cytotrophoblasts covering the tips of the villi have differentiated into extravillous trophoblasts that invade decidua and its vasculature (Benirschke and Kaufmann, 2000) (**Figure 2B**). This process attaches placenta to the uterus and provides the blood supplies for the fetus.

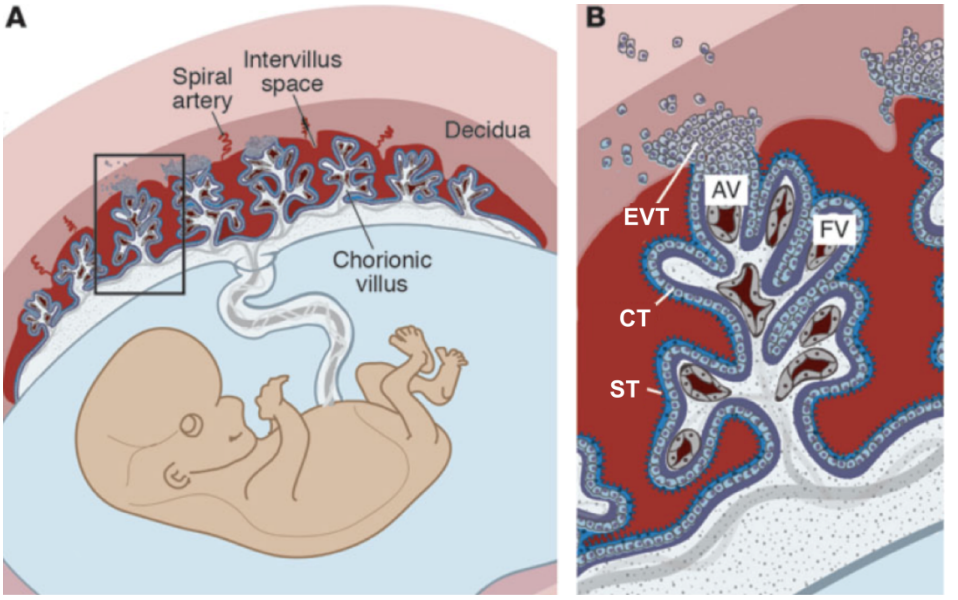

Figure 2. Anatomy of human placenta. A. Intervillous space between the chorion and the decidua is packed with chorionic villi and filled with maternal blood. **B.** The floating villi (FV) of placenta are covered with a continuous double-layer of trophoblast cells. The outer layer of multinuclear syncytiotrophoblast (ST) is in direct contact with maternal blood. The inner layer of mononuclear cytotrophoblast (CT) represents the progenitor population of trophoblast cells. Anchoring villi (AV) are attached to the uterus by invasive extravillous trophoblasts (EVT) (Maltepe *et al*., 2010).

2.1.2. Functions of human placenta

Placenta performs several critical functions to fulfil the increasing demands of the developing fetus while assuring maternal adaption over gestation. The main functions of placenta are the following:

1. *Transport and metabolism*. Maternal blood in the intervillous space is in direct contact with placental villi that facilitate the transport of water, nutrients (glucose, amino acids, lipids, minerals, vitamins), oxygen, and waste products across the placental membrane (Gude *et al*., 2004). Different molecules transfer either by active placental transporters or passively (Hay, 1994). Besides mediating nutrients and oxygen between the two organisms, placenta also requires these compounds for generation of metabolic products.
2. *Hormonal regulation*. Placenta produces molecules employed both locally and distally to coordinate the maternal-fetal dialogue during pregnancy. Placental syncytiotrophoblast acts as an endocrine organ that produces and secretes several proteins, hormones, cytokines into maternal and fetal organisms to maintain pregnancy and prepare for parturition and lactation. One of the first hormones produced by placenta is the luteotrophic human

chorionic gonadotropin (HCG, see below) that stimulates ovaries to synthesize the essential steroids in pregnancy (Cole, 2010; 2012a). The human growth hormone (GH) and placental lactogen (PL; also known as human chorionic somatomammotropin hormone, CSH) regulate maternal and fetal metabolism as well as fetal growth and development (Handwerger and Freemark, 2000).

3. *Immunological barrier*. As placenta is genetically identical to the fetus and distinct from the mother, its crucial task is to trigger the maternal immune system to suppress a response against the developing fetus (Moffett and Loke, 2006).
4. *Protection*. Placenta protects the fetus against xenobiotics, infections, and maternal diseases. For example, the maternal immunoglobulin G antibodies are transported to the fetus to provide passive immunity for the newborn (Simister, 2003). Placenta also forms a barrier against transmission of bacteria from the mother to the fetus.

2.1.3. Specific features of primate placental evolution

2.1.3.1. Structural evolution of placenta in mammals

Placenta is one of the most variable of all mammalian organs, as it has evolved rapidly in the mammalian lineage after arising about 100–150 million years ago (Springer *et al*., 2003). The structure of placenta as well as the length of gestation, number of offspring and body mass of the newborn varies greatly across placental mammals (Wildman, 2011). As the types of placenta are distributed within the mammalian family but are not consistent with the known phylogeny (Enders and Carter, 2004), the debate over placental evolution among mammals is still ongoing.

The basic classification of placental types considers its invasiveness into the uterus, explained by the number of cellular layers separating the maternal blood from the fetal blood. The simple non-invasive epitheliochorial placenta consists of three maternal layers (endothelium, connective tissue, and endometrial endothelium) and three fetal layers (endothelium, chorionic connective tissue, and trophoblast) that separate maternal and fetal blood streams. In endotheliochorial placenta, deeper invasion of embryo into endometrium has eroded endometrial endothelial cells and placental trophoblast cells are in direct contact with maternal capillary endothelial cells (**Figure 3A, B**) (Benirschke *et al*., 2006). Such placental types are common for ruminants and carnivores, respectively. Humans, anthropoid primates and rodents have evolved deeper, more efficient placental invasion mechanisms and hemochorial placentation systems where all three maternal cell layers are degraded and trophoblasts are in direct contact with maternal blood (**Figure 3C**) (Benirschke *et al*., 2006; King, 1993). Such structure allows maximum transport of nutrients to the fetus and is considered necessary to support the development of large fetal brains in humans (Cole, 2009).

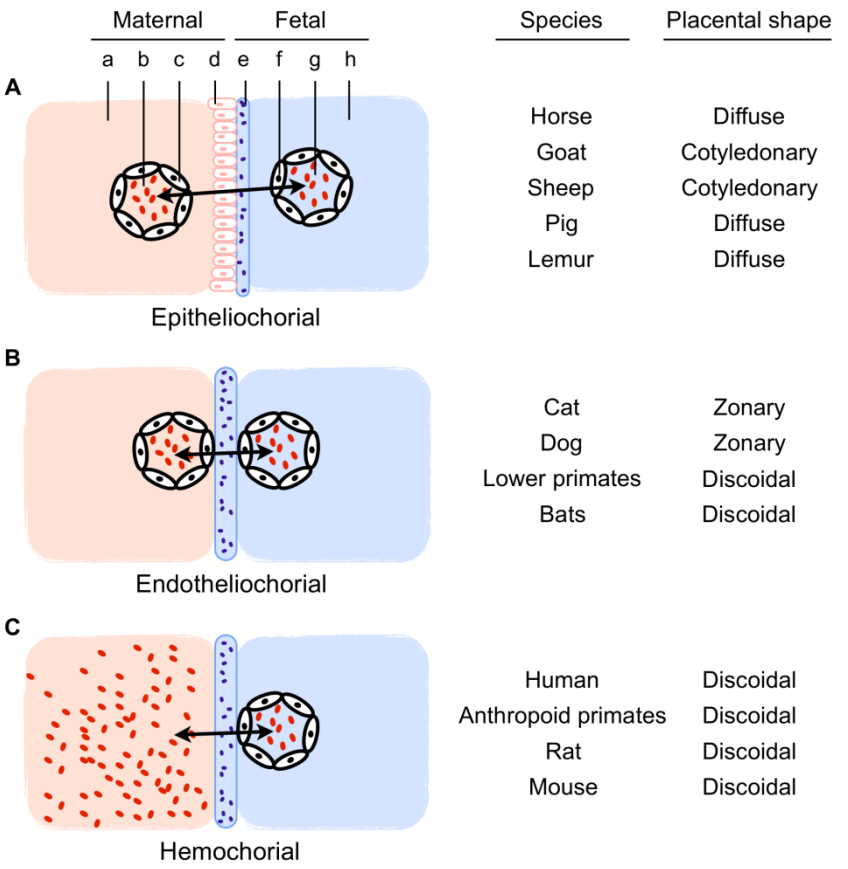

Figure 3. Schematic representation of the types of placentation in mammals. A. In epitheliochorial placenta the fetal trophoblast cells and maternal blood are separated by three layers of tissue. **B.** In endotheliochorial placenta the maternal blood is separated from the fetal trophoblast by the maternal capillary endothelium. **C.** In hemochorial placenta the maternal blood cells are in direct contact with the fetal trophoblast cells. (a) Maternal connective tissue, (b) maternal blood, (c) maternal endothelium, (d) maternal endometrial endothelium, (e) fetal trophoblast, (f) fetal endothelium, (g) fetal blood, (h) fetal chorionic connective tissue. The data were assembled from Benirschke *et al*., 2006.

Besides invasiveness, the shape of placenta and distribution of contact sites between fetal membranes and endometrium has diverged between species. For example, ruminate gestation is characterized by diffuse placenta that completely surrounds the fetus or cotyledonary placenta with multiple attachment sites in uterine wall. Carnivores have zonary shaped placentas that form a band around the fetus. The discoidal placenta in humans, anthropoid primates, and rodents has a round, flat, disk-like structure (Benirschke *et al*., 2006).

2.1.3.2. Accelerated evolution of placental genes in the primate lineage: novel gene families, microRNAs, and retroviral infection

Besides the anatomical similarities between primate placentas, placental genes exhibit rapid evolution in the primate lineage. Several novel gene families regulating the developmental, reproductive and immune system functions have uniquely expanded in the primate placenta through gene duplication events (Rawn and Cross, 2008). Such unique primate placenta-specific loci include genes important for embryo implantation and formation of invasive hemochorial placenta. For instance, the chorionic gonadotropin β-coding genes (*CGB*) have arisen from the ancestral luteinizing hormone β-coding (*LHB*) gene (see below) (Maston and Ruvolo, 2002). In addition, anthropoid primates are the only species known to produce the corticotropin-releasing hormone (CRH) that regulates the length of gestation and timing of parturition (McLean *et al*., 1995; Robinson *et al*., 1989), as well as placental galectins that mediate maternal-fetal immune response (Than *et al*., 2009). Furthermore, cadherins and killer-cell immunoglobulin-like receptors (KIRs) that regulate maternal-fetal interactions and immunological reactions are under selective pressure in the primate placenta (Older Aguilar *et al*., 2010; Summers and Crespi, 2005). Several placental growth hormones and lactogens have also evolved in the primate linage in sequential gene conversion and duplication events from the ancestral pituitary-expressed *GH* gene (Li *et al*., 2005; Papper *et al*., 2009; Petronella and Drouin, 2011). Surprisingly, the placental lactogens in rodents originate from the prolactin gene instead (Soares *et al*., 1998), providing evidence of a parallel evolution of placenta-specific genes in human and rodent lineages (**Table 1**). Another example is the family of pregnancy-specific glycoprotein (*PSG*) genes where the ancestral gene is thought to be common to both primates and rodents, but subsequent gene duplications have arisen independently between species (**Table 1**) (McLellan *et al*., 2005; Rudert *et al*., 1989).

Thus, accelerated parallel evolution of placental genes appears to be fundamental in mammalian placenta. It is still not clear whether the novel genes first expanded in the genome and then gained their distinct functions, or the ancestral genes had gained multiple functions by themselves before the duplication events (Maston and Ruvolo, 2002). However, the placental regulatory molecules that determine maternal and fetal metabolism support the maternal-fetal conflict hypothesis for placental evolution (Haig, 1993; 2008). Accelerated evolution of placental genes in primates may associate with the development of hemochorial placenta that allows direct transport of placentally derived large molecules into maternal blood stream where they are further transported to the location of action (King, 1993).

Although most of the ancient endogenous retroviral sequences in the mammalian genomes are silenced, there are still some sequences that can produce functional proteins. A number of human endogenous retroviral genes are known

to be expressed in placenta and be conserved in the primate lineage. For example, the Syncytin-1 and Syncytin-2 envelope proteins of retroviral origin lead the fusion of cytotrophoblast cells to multinucleated syncytiotrophoblast. Syncytin genes are highly conserved and found in all anthropoid primates, referring to strong evolutionary selection (Blaise *et al*., 2003). However, rodents have independently acquired their own Syncytin genes (*Syncytin-A*; *Syncytin-B*) from a different endogenous retrovirus family (Dupressoir *et al*., 2005).

Finally, several novel microRNA gene families have been subject to rapid evolution, contributing to the functional diversity of placenta in the primate lineage. Examples include the microRNA clusters on chromosome 19 (C19MC, comprised of 54 tandem repeated microRNAs) and on chromosome 14 (the C14MC cluster with 34 microRNAs) (Bentwich *et al*., 2005; Seitz *et al*., 2004). MicroRNAs are small single-stranded RNA molecules (~19–22 bp) that function as transcriptional and post-transcriptional modulators of gene expression (Chen and Rajewsky, 2007). The expression of microRNAs from the clusters C19MC and C14MC is under epigenetic regulation and has been largely found in placenta but also in brain (Noguer-Dance *et al*., 2010; Seitz *et al*., 2004). The precise placental roles of these microRNAs are still unknown, although C19MC microRNAs are thought to participate in placental-maternal signalling (Donker *et al*., 2012).

Table 1. Examples of parallel evolution of placenta-specific genes in human and rodent lineages (Rawn and Cross, 2008)

Gene family	Species	Number of genes	
		All	Placenta-specific
Prolactin	Human	1	0
	Mouse	23	22
	Rat	24	23
Pregnancy-specific beta-1 glycoprotein	Human	11	11
	Mouse	17	17
	Rat	8	8
Growth hormone	Human	5	4
	Mouse	1	0
	Rat	1	0
Chorionic gonadotropin β	Human	6	6
	Mouse	0	0
	Rat	0	0

2.1.4. Primate-specific placental hormone chorionic gonadotropin

2.1.4.1. Properties and role of HCG (see also Ref. I)

The placenta-specific hormone chorionic gonadotropin has only been found in humans, some primates and equine (Maston and Ruvolo, 2002; Sherman *et al*., 1992). HCG is a key molecule that determines the success of early pregnancy (Srisuparp *et al*., 2001). This glycoprotein hormone is composed of a common α-subunit shared with all gonadotropins (CG – chorionic gonadotropin, LH – luteinizing hormone, FSH – follicle stimulating hormone, TSH – thyroid stimulating hormone) as well as a unique β-subunit that confers biological function and is encoded by a set of duplicated *CGB* genes (Pierce and Parsons, 1981). HCG production starts already in the pre-implantation embryo. The hormone is transported to the maternal bloodstream during implantation where it reaches its peak at the 9–11th week of gestation (Stenman *et al*., 2006). The circulation half-life of HCG is about 36 hours. Detection of HCG in urine or serum is widely used to diagnose early pregnancy (Cole, 2012c). Importantly, the final HCG level is determined by the expression of its β-subunit whereas the α-subunit is always expressed in excess (Miller-Lindholm *et al*., 1997).

The major function of HCG in human pregnancy is to drive hemochorial placentation (Cole, 2012a). HCG prevents the regression of *corpus luteum* in the ovary. It stimulates continued production of progesterone during early gestation until the mass of formed syncytiotrophoblast is sufficient to produce the progesterone independently (Hay, 1988). HCG also facilitates angiogenesis and vasculogenesis in the uterine vasculature at implantation and placentation (Licht *et al*., 2001; Zygmunt *et al*., 2003), and stimulates the differentiation of cytotrophoblasts into syncytiotrophoblasts (Shi *et al*., 1993). In addition, HCG drives the immunological adaption during pregnancy and protects the fetus against maternal immune rejection (Akoum *et al*., 2005; Wan *et al*., 2007). During the second and third trimester, HCG prevents myometrial contractions and maintains the endometrial lining of the uterus (Edelstam *et al*., 2007; Eta *et al*., 1994). In addition, HCG plays a role in male fetal sexual differentiation, stimulating fetal testosterone synthesis in the testicular Leydig cells (O'Shaughnessy *et al*., 2006). The hormone also promotes the growth and differentiation of fetal organs during pregnancy (Goldsmith *et al*., 1983; Rao and Lei, 2007).

Although HCG concentrations during pregnancy show high inter-individual variation in maternal serum (Cole, 2012b) (**Figure 6 in Ref. I**), abnormal hormone expression associates with pregnancy complications (Buyalos *et al*., 1992; Stenman *et al*., 2006). Moreover, non-pregnancy related expression of HCG has been used as marker for trophoblastic and non-trophoblastic malignancies (Cole, 2010; Cole *et al*., 1983; Stenman *et al*., 2004). In pregnancy complications, the first trimester level of HCG is elevated in cases of fetal chromosomal anomalies such as trisomy 21 (Bogart *et al*., 1987) whereas the HCG level is decreased in cases of trisomy 18 (Jauniaux *et al.*, 2000). High levels of

HCG may also indicate a molar pregnancy or the risk for preeclampsia (Gurbuz *et al*., 2004). Low serum HCG and abnormal expression pattern of the hormone during the first trimester of pregnancy may refer to maternal susceptibility to spontaneous abortion, ectopic pregnancy (EP) or failure of assisted reproduction procedure (Chi *et al*., 2010; Letterie and Hibbert, 2000; Poikkeus *et al*., 2002; Tong *et al*., 2006).

2.1.4.2. Evolution of HCG beta subunit coding genes among primates

Understanding the evolution and function of *HCGβ-coding* genes has great importance in the context of placental biology and evolution, but also in the context of human evolution. Interestingly, the *CGB* gene family has specifically evolved in the anthropoid lineage, and the number of functional *CGB* genes is the highest in the human genome compared to other primates.

The primate-specific *CGB* genes arose approximately 55–35 million years ago, following the duplication of the ancestral *LHB* gene and before the divergence of New World and Old World monkeys (Bailey *et al*., 1991; Maston and Ruvolo, 2002) (**Figure 4A**). The *CGB* genes appear to be co-evolved with the hemochorial placenta (King, 1993). The first *CGB* gene appeared in the lineage of New World Monkeys, whereas in the Old World Monkeys, great apes, and the human lineage, additional gene duplication events have led to the further expansion of the *CGB* gene family (Hallast and Laan, 2009; Maston and Ruvolo, 2002) (**Figure 4A**). Comparison of the entire *LHB/CGB* gene cluster between human and chimpanzee revealed parallel independent duplication events in the two species, resulting in a discordant number of *CGB* genes in humans (n=6) and chimpanzees (n=5) (**Figure 4A**) (Hallast *et al*., 2008). Most recently, the novel protein non-coding *CGB1* and *CGB2* genes were duplicated in the lineages of humans and African great apes; these are strongly conserved between human and chimpanzee (Hallast *et al*., 2007) (**Figure 4A**). The two genes were formed by the insertion of a DNA element containing *snaR-G* gene into their 5' ends, drastically modifying the encoded proteins. A recent study suggested that the *CGB* genes have an important role in the primate male reproductive system (Parrott *et al*., 2011).

Chorionic gonadotropin is also found in equines but its evolution differs greatly from the primates. No duplication event of the *LHB* gene is apparent in the equine genome. Both, equine LH and CG are encoded by the same common α-subunit and the equine *LHB* gene (Sherman *et al*., 1992). The difference in function is achieved through different glycosylation, with the equine CG hormone being more glycosylated (Sherman *et al*., 1992). Among other mammals, *CGB* genes are not present in mice, rats, cows, pigs and sheep (Brown *et al*., 1993; Ezashi *et al*., 1990; Jameson *et al*., 1984; Kumar and Matzuk, 1995; Virgin *et al*., 1985).

The human *LHB/CGB* gene cluster is located in a 50 kb region on chromosome 19q13.32 and consists of a tandem of one homologous *LHB* and six *CGB*

genes (Hallast *et al*., 2005; Policastro *et al*., 1986) (**Figure 4C**). The *HCGβ*-coding genes (*CGB*, *CGB5*, *CGB7* and *CGB8*) have 97–99% of DNA sequence identity, whereas their identity with the functionally divergent *LHB* gene is 92–93% (Bo and Boime, 1992; Hallast *et al*., 2005) (**Figure 4B**). Non-protein coding pseudogenes *CGB1* and *CGB2* are 85% similar to the *HCGβ*-coding genes (**Figure 3B**). In addition to high DNA sequence similarity, the *LHB/CGB* genomic region is also characterized by extremely high GC nucleotide content (≥55% compared to ~40% of genome average), abundance of CpG islands and high repeat content (Hallast *et al*., 2005) (**Figure 4D, E**).

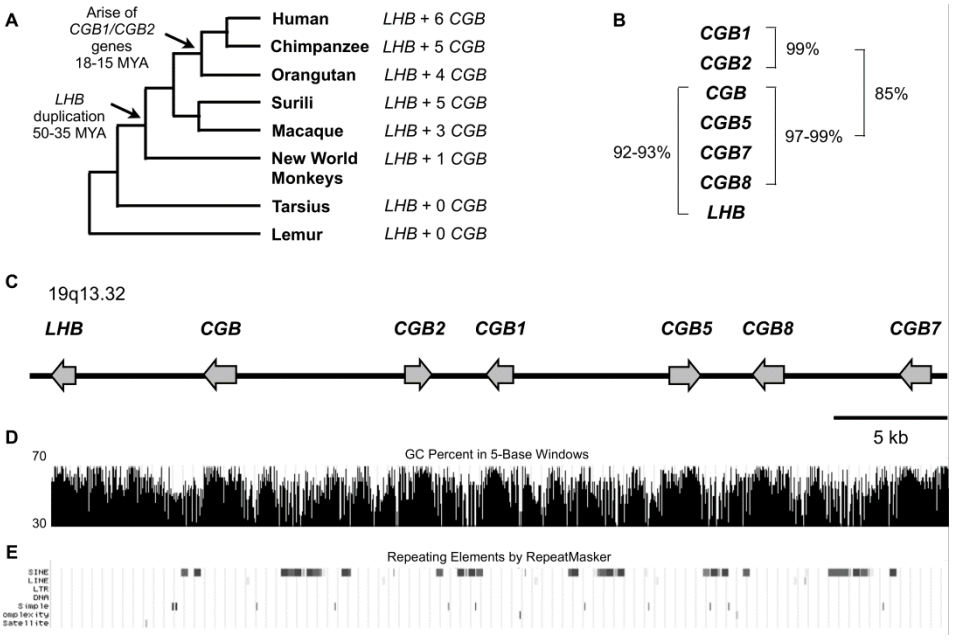

Figure 4. Evolution and genomic organization of the human *LHB/CGB* gene cluster. A. Several duplication events of the ancestral *LHB* gene have led to the rise of *CGB* genes in the primate lineage (adapted from Maston and Ruvolo, 2002) **B.** Six human *CGB* genes share up to 99% of sequence identity and **C.** are located in chromosome 19q13.32 in the common gene cluster with *LHB* gene, covering a genomic region of about 50 kilobases. **D.** The genomic region of *LHB/CGB* cluster is defined by high CG content (≥55%) and **E.** high number of repeats (data from USCS genome browser). MYA, million years ago.

2.2. Gene expression in human placenta

The normal function of placenta depends on the proper growth and development of its structural components, requiring finely tuned transcriptional regulation of placental genes during gestation. Unique features characterize placental gene expression. First, although the placental genome is identical to the genome of

the fetus, the regulation of gene expression differs greatly between the two. Second, placental transcription is dynamic in nature and it changes over gestation in a time-dependent manner. Third, placental gene expression regulates fetal development, maternal metabolism as well as placental development and function during pregnancy. Last but not least, gene expression in placenta also programs its own death at the end of third trimester. Several earlier studies have investigated placental gene expression and linked its alterations to placental pathologies of development, maternal metabolism and the immune system. Yet, complete characterisation of placental gene regulation over the course of normal pregnancy is an important prerequisite for understanding placenta-related pregnancy complications.

2.2.1. Gene expression of primate-specific placental duplicate genes

2.2.1.1. HCGβ-coding genes

The expression of *HCGβ-coding* genes in primates is mainly restricted to placenta (Jameson *et al*., 1986). Among the six duplicate genes, the *HCGβ*-coding genes *CGB8*, *CGB5* and *CGB* have the highest placental expression, while the pseudogenes *CGB1* and *CGB2* are expressed at much lower level (Miller-Lindholm *et al*., 1997; Rull and Laan, 2005). The majority of *HCGβ* transcription in the hypoxic first trimester placenta is driven by villous cytotrophoblast cells, whereas the syncytiotrophoblast becomes the major source of transcription after the maternal blood has entered the placental intervillous space (Cocquebert *et al*., 2012). Although sufficient transcription of *HCGβ*-genes is crucial for the final hormone HCG level in gestation, the transcription of single *HCGβ-coding* genes varies greatly between individuals and populations (Bo and Boime, 1992; Miller-Lindholm *et al*., 1997; Rull and Laan, 2005). However, the maintenance of normal pregnancy is determined by the total expression of *HCGβ*-coding genes rather than the expression of individual *HCGβ* genes. Of note, increased total *HCGβ* expression also serves as a molecular biomarker for malignant tumours (Stenman *et al*., 2004).

2.2.1.2. GH/CSH-coding genes

The locus coding for the human *Growth Hormone/Chorionic Somatomammotropin* (*GH/CSH*) on chromosome 17q22–24 consists of highly similar pituitary-expressed postnatal gene (*GH1*) and four placentally expressed duplicate genes with sequence identity of 92–98% (*GH2*, *CSH1*, *CSH2*, and *CSHL1*) (George *et al*., 1981; Sedman *et al*., 2008). *GH2* encodes the human placental growth hormone (PGH), while the *CSH1* and *CSH2* genes encode the placental lactogen (PL). Both hormones regulate fetal growth and development, and are

required for the adaptation of the maternal metabolism to pregnancy (Alsat *et al*., 1998; Freemark, 2006). The placental gene *CSHL1* is not known to be protein coding. Despite structural similarity, the four placental *GH/CSH* genes show considerable heterogeneity and variability, especially in transcriptional regulation and alternative splicing, resulting different protein isoforms (MacLeod *et al*., 1992; Sedman *et al*., 2008).

Aberrant mRNA expression of placental *GH/CSH* genes is associated with several pregnancy complications, manifested in altered maternal metabolism and fetal growth. For example, decreased placental expression of *GH/CSH* genes has been found in cases of restricted fetal growth whereas increased placental expression of the same genes is associated with elevated fetal birth weight (Männik *et al*., 2010). Additionally, decreased and increased mRNA expression of *GH/CSH* genes was found in cases of preeclampsia and gestational diabetes mellitus, respectively (Mannik *et al*., 2012).

2.2.2. Placental transcriptome analysis in normal and complicated pregnancy

2.2.2.1. Gestational dynamics of placental gene expression in normal pregnancy

Placental gene expression is continuously adapted during pregnancy to facilitate the requirements of the developing fetus and changes in maternal metabolism. During early pregnancy, normal trophoblast development determines successful implantation and formation of the maternal-fetal interface. Mid-gestation placenta supports proportional fetal growth, organ development, and fine-scale differentiation, as well as continuous adaption of maternal metabolism. During the third trimester of pregnancy, placenta provides increasing amounts of nutrients for the growing fetus. However, despite the great importance of placenta in mediating the rapid physiological changes in pregnancy, data on the temporal dynamics of human placental gene expression are limited.

Placental gene expression in normal pregnancies across different gestational ages has been addressed in three studies. Winn *et al.* compared transcription between term and mid-gestation placentas (Winn *et al*., 2007) whereas Sitras *et al.* focused on changes between first and third trimester placental samples (Sitras *et al*., 2012). Mikheev *et al.* compared the transcriptional profiles of first and second trimester samples with term pregnancy samples (Mikheev *et al*., 2008). All three studies observed profound changes in global gene expression at the end of pregnancy. The authors observed alterations in nearly 25% of placental transcriptome and coordinated regulation of pathways to acquire rapid changes in placental function. Regulators of cell cycle, differentiation and motility, angiogenesis, macromolecule biosynthesis, and metabolism were the major gene groups up-regulated in early pregnancy, while genes involved in apoptosis, inflammatory process, signal transduction, and stress response were

highly expressed at term (Mikheev *et al*., 2008; Sitras *et al*., 2012; Winn *et al*., 2007). However, none of the studies described gene expression in first and second trimester placentas of normal human pregnancy.

Besides the temporal gene expression studies above, two genome-wide transcriptional comparisons of placentas from C-sections and labours have revealed insights into the labour process and its effects on placental gene expression (Cindrova-Davies *et al*., 2007; Lee *et al*., 2010). Genes involved in stress response, immune response, cell death, coagulation, and blood vessel development showed the largest transcriptional differences between placental samples from labour and non-labour C-sections. These functional categories are considered to be closely associated with the inflammatory response that characterizes labour. In addition, the transcriptional changes in early pregnancy, such as differentiation from villous to extravillous trophoblast, have been examined using whole genome microarrays (Apps *et al*., 2011). The authors found that proper differentiation is critical for trophoblast invasion and relates to remodelling of decidual spiral arteries. Failure to transform arteries leads to poor trophoblast invasion, resulting in inadequate placental perfusion that in turn induces the development of pregnancy complications such as preeclampsia, fetal growth restriction or recurrent miscarriage.

Studies addressing the temporal changes in gene expression during normal pregnancy are especially important, as these will provide insight into the healthy gene regulation in the developing placenta. The results of such reports will serve as a reference to compare gene expression aberrations in placentas from complicated pregnancies.

2.2.2.2. Altered placental gene expression in pregnancy complications

As the healthy placenta carries a central role in the successful outcome of pregnancy, studies into altered placental gene expression in pregnancy complications have gained increasing interest. Whole-genome transcription studies of pregnancy complications aim to identify molecular networks and transcriptional mechanisms underlying placental pathologies, and discover novel non-invasive biomarkers and potential drug targets. So far, placental microarray studies have addressed gene expressional changes in either early or late pregnancy complications (examples are shown in **Table 2**).

Early pregnancy complications such as miscarriage, ectopic and molar pregnancy are most commonly characterised by aberrant expression of genes regulating implantation, maternal-fetal immunological functions, apoptosis and angiogenesis (Kim *et al*., 2006; Kokawa *et al*., 1998; Nakashima *et al*., 2012). Miscarriage is the most common early pregnancy complication, characterised by severely impaired development of placental-decidual interface with reduced maternal blood flow and oxidative stress (Jauniaux et al., 2006). Although considerable effort has been taken to map the genes involved in pathogenesis of miscarriage, no high-confidence predictive biomarkers have been found in

gene-focused association studies (Rull *et al*., 2012). However, recent whole-genome differential gene expression analysis of recurrent miscarriage (RM) placentas revealed increased placental mRNA expression of the gene encoding *TNF-related apoptosis-inducing ligand* (*TRAIL*) (Rull *et al*., 2013). In addition, significantly higher maternal serum concentration of soluble TRAIL in cases of RM, unpredicted miscarriage, and tubal pregnancy refers to TRAIL's potential of being a predictive biomarker for early pregnancy complications (Rull *et al*., 2013).

Impaired trophoblast invasion and vascularisation in early pregnancy are also associated with term complications such as preeclampsia (PE) and fetal growth restriction (FGR), referring to their common etiological and molecular background (Nishizawa *et al*., 2011). Trophoblast invasion is sufficient for early placentation but too shallow to complete the transformation of the arterial utero-placental circulation, leading to hypoxic intrauterine environment later in pregnancy (Jauniaux et al., 2006). Founds *et al.* compared first trimester chorionic villous samples of women who developed PE at the end of gestation to samples of uncomplicated pregnancies, and found several differentially expressed genes associated with inflammation, immune regulation and cell motility (Founds *et al*., 2009). Another study suggested that PE and FGR complications involve similar gene regulatory pathways, as most of the genes up-regulated in PE placentas at term are also up-regulated in FGR placentas (**Table 2**) (Nishizawa *et al*., 2011). However, early detection methods of these pregnancy complications remain limited.

Recent genome-wide transcriptome analyses of placental gene expression in term pregnancy complications have associated several genes to complicated pregnancies (**Table 2**). Interestingly, differential expression of some genes is common to several complications, suggesting that these may be interpreted as general biomarkers of malfunctioning placenta. For example, significantly increased expression of genes such as *FLT1* (Fms-related tyrosine kinase 1), *LEP* (leptin), *PAPPA2* (Pappalysin-2) is found in placentas corresponding to preeclampsia, gestational diabetes mellitus (GDM), and intrauterine growth restriction (IUGR) (**Table 2**).

Table 2. Recent genome-wide transcription studies of human placenta from normal and complicated pregnancies.

Study	Method of analysis	Number of samples	Major findings in complicated pregnancies
Recurrent miscarriage (RM)			
(Rull *et al*., 2013)	Affymetrix HG-U133 Plus 2.0 Gene Chip; RT-qPCR; ELISA;	1st trim RM (*n*=13); 1st trim control (*n*=6)	– 30 differentially expressed transcripts (FDR<0.05); – ↑ *TRAIL*, *S100A8*; – Maternal serum sTRAIL is a potential predictive biomarker for RM
Hydatidiform mole			
(Kim *et al*., 2006)	MacArrayTM Express Human 10 K Chip	1st trim mole (*n*=4); 1st trim control (*n*=4)	– 91 genes up-regulated, 122 genes down-regulated; – ↑ *TLE4*, *CAPZA1*, *PRSS25*, *RNF130*, and *USP1*; – ↓ *ELK3*, *LAMA3*, *LNK*, *STAT2*, and *TNFRSF25*
Preeclampsia (PE)			
(Lapaire *et al*., 2012)	Affymetrix Human Gene 1.0 ST array; RT-qPCR ELISA	3rd trim PE (*n*=9) 3rd trim control (*n*=7)	– 896 differentially expressed genes; – ↑ *β-hCG*, *HTRA4*, *LHB*; – ↓ *NOX4*; – Increased maternal serum β-hCG and LHB
(Tsai *et al*., 2011)	Illumina Human6-v2 BeadArrays; RTq-PCR	3rd trim PE (*n*=23) 3rd trim control (*n*=37)	– 2109 differentially expressed genes (FDR<0.05); – ↑ *LEP*, *FLT1*, *PAPPA2*, *ENG*, *INHA*, *SIAE*; – ↓ *CD4*
(Sitras *et al*., 2009b)	30K Human Genome Survey Microarray v.2.0	3rd trim PE (*n*=19) 3rd trim control (*n*=21)	– 213 genes up-regulated, 82 genes down-regulated (fold-change≥2 and p≤0.01); – Different gene expression between early- and late-onset preeclampsia; – ↑ *FLT1*, *INHBA*, *PAPPA2*, *CGB5*, *LEP*; – ↓ *BMP5*, *BHLHB3*, *PDGFD*

Study	Method of analysis	Number of samples	Major findings in complicated pregnancies
Intrauterine growth restriction (IUGR)			
(Struwe *et al*., 2010)	Affymetrix HG-U133 Plus 2.0 Gene Chip; RT-qPCR	3rd trim IUGR (*n*=20) 3rd trim control (*n*=20)	– 132 up-regulated genes, 25 down-regulated – ↑ *LEP*, *CRH*, *IGFBP-1*,
PE and fetal growth restriction (FGR)			
(Nishizawa *et al*., 2011)	Affymetrix Human Exon 1.0 ST Array	3rd trim PE (*n*=8) 3rd trim FGR (*n*=8) 3rd trim control (*n*=8)	– 62 common differentially expressed genes for FGR and PE; – ↑ *FLT1*, *ENG*, *HCGβ*, *LEP*
Gestational diabetes mellitus (GDM)			
(Enquobahrie *et al*., 2009)	Operon's Human Genome Array Ready Oligo Set version 2.1; RT-qPCR	3rd trim GDM (*n*=19) 3rd trim control (*n*=21)	– 66 up-regulated genes (fold change≥3; FDR≤0.1); – ↑ *AQP3*, *LEP*, *FLT1*, *ADFP*, *CEBPA*, *MIF*

2.2.3. Epigenetic control of placental gene expression

According to the definition, epigenetics refers to heritable changes in gene expression that are not manifested in the DNA sequence (Jones and Takai, 2001). As DNA is largely fixed in all cells of an organism throughout its life, epigenetic mechanisms carry an important role in regulating gene expression to produce different organs and cell types (Byun *et al*., 2009). Moreover, epigenetic patterns can be passed from generation to generation, change over time, and are sensitive to environmental exposures (Aguilera *et al*., 2010; Christensen *et al*., 2009). Epigenetic regulation in placenta and other tissues controls transcription at three levels: DNA (DNA methylation), protein (histone modifications) and RNA (long non-coding RNAs) (Nelissen *et al*., 2011). DNA methylation represses transcription by adding methyl groups to the 5th position of a cytosine next to guanine at genomic regions of high cytosine and guanine content (CpG islands) (Bird, 1986). Histone modifications either repress (e.g. methylation of lysine and arginine residues of histones) or activate (e.g. acetylation of lysine residues) transcription through modifications of chromatin structure and changes in DNA accessibility (Rice and Allis, 2001; Strahl and Allis, 2000). Finally, long non-coding RNAs (lncRNAs) participate in epigenetic regulation through parent-of-origin and tissue specific silencing of gene expression (Amaral *et al*., 2010; Mohammad *et al*., 2012). Genomic imprinting affects gene dosage through repression of the imprinted allele (Ferguson-Smith and Surani, 2001) and the expression of imprinted genes is parent-of-origin specific and monoallelic (Reik and Walter, 2001).

Epigenetic regulation of gene expression is especially important in modulating placental gene activity and chromosome structure. Notably, more than 70% of epigenetically regulated genes are expressed in placenta (Diplas *et al*., 2009; Nelissen *et al*., 2011). Furthermore, the epigenetic mechanism of imprinting is thought to have co-evolved with the chorioallantoic placenta in the mammalian lineage, to provide more complex regulation of placental genes over gestation (Haig, 1996). The hypothesis of parental genome conflict describes the origin of genomic imprinting and postulates that the paternal genome tries to maximise fetal resource acquisition from the mother while the maternal genome aims to balance its resources to the offspring (Haig, 1996; Moore and Haig, 1991). These observations and hypotheses underline the importance of epigenetic regulation in placenta.

However, the epigenetic marks are not constantly maintained throughout an individual's life. The primordial germ cells and the pre-implantation embryo undergo genome-wide epigenetic programming during development. First, the methylation of primordial germ cells is completely erased during migration towards the forming genital ridges, followed by global gender-specific *de novo* establishment of methylation (Ewen and Koopman, 2010; Hajkova *et al*., 2002). After fertilisation, a second wave of genome-wide de-methylation and subsequent *de novo* re-methylation takes place in the pre-implantation embryo (Mayer *et al*., 2000b; Reik *et al*., 2001). By the blastocyst stage, both maternal

and paternal chromosomes have undergone progressive demethylation (Li, 2002; Rougier *et al*., 1998). Only the differential methylation of imprinted genes remains unaffected (Morgan *et al*., 2005). DNA methylation patterns in the developing embryo are re-established after implantation (Li, 2002). Such epigenetic programming of germ cells and embryos may be required to acquire the essential characteristics of immorality and totipotency in development (Sasaki and Matsui, 2008; Surani *et al*., 2007). In addition, imprinted genes are not randomly distributed across the genome but tend to occur in clusters and are often controlled by a shared imprinting control region (Wagschal and Feil, 2006; Wood and Oakey, 2006). Two general features of the DNA sequence environment of imprinted genes are known: first, the regions are unusually rich in CpG islands, and second, direct repeats are common to CpG islands (Reik and Walter, 2001).

2.2.3.1. Targeted studies of epigenetically regulated genes in normal and complicated human placenta

So far, approximately 80 imprinted human genes and 130 mouse genes have been described (Jirtle, 2012; Morison *et al.*, 2001). However, the overlap of imprinted genes between the two species is small (~50%) and imprinting is not always conserved. The lack of conservation in imprinting may relate to the differences of placentation and pregnancy between humans and mice (Carter, 2007; Enders, 2009).

The majority of imprinted genes are expressed in placenta but also in the fetus, postnatal brain and endocrine tissues (Bressan *et al*., 2009; Coan *et al*., 2005; Davies *et al*., 2005). The main functions of imprinted genes involve the regulation of prenatal growth of embryo and/or placenta, and the regulation of metabolic pathways and higher brain functions (Constancia *et al*., 2004; Morison *et al*., 2005; Reik and Walter, 2001; Tycko, 2006). Both the accurate establishment of genomic imprints as well as their correct maintenance are essential for normal fetal and placental development, demonstrated in diseases caused by aberrant imprinting or animal knockout experiments (**Table 3**) (Malassine *et al*., 2003; Toppings *et al*., 2008). Loss of imprinting (LOI) is the gain of function from the silenced parental allele that affects gene dosage. Besides imprinting, gene silencing through gain of methylation of a non-imprinted allele affects gene dosage, potentially resulting in a disease condition (Novakovic and Saffery, 2012). Diseases associated with imprinting include various cancers, disorders of growth and metabolism, and disorders in neuronal development, cognition, and behaviour, including certain major psychiatric disorders (Wilkins and Ubeda, 2011).

Table 3. Examples of conserved imprinted genes in human and mouse, their aberrant expression in human disease and mouse knockout phenotypes.

Gene	Physiological function	LOI, increased or decreased expression	Mouse KO phenotype	References
Paternal allele expressed				
PEG10 (Paternally expressed gene 10)	Placental development; Adipocyte differentiation	Hepatocellular carcinoma; IUGR (increased expression)	Embryonic lethality; Incomplete placenta formation	(Ono *et al*., 2006) (Smallwood *et al*., 2003) (Gao *et al*., 2010) (Hishida *et al*., 2007) (Diplas *et al*., 2009)
MEST (Mesoderm specific transcript homolog)	Angiogenesis in trophoblast tissue and decidua	IUGR (decreased expression)	Embryonic and placental growth retardation; Aberrant maternal care	(Mayer *et al*., 2000a) (Lefebvre *et al*., 1998) (McMinn *et al*., 2006)
PEG3 (Paternally expressed gene 3)	Apoptosis, TNF-signalling pathway	Glioblastoma	Embryonic and placental growth retardation, aberrant maternal care	(Relaix *et al*., 2000) (Li *et al*., 1999) (Otsuka *et al*., 2009)
MAGEL2 (MAGE-like 2)	Cell cycle; Differentiation; Apoptosis; Nervous system development; Circadian rhythm regulation		Abnormal behaviour in novel environments; Reduced fertility; Neonatal mortality; Altered suckling activity	(Mercer *et al*., 2009) (Mercer and Wevrick, 2009) (Schaller *et al*., 2010)

Gene	Physiological function	LOI, increased or decreased expression	Mouse KO phenotype	References
IGF2 (Insulin-like growth factor 2)	Nutrient supply regulation; prenatal growth regulation	Beckwith-Wiedemann Syndrome; Silver-Russell Syndrome; Wilms' tumor	Reduced placental growth, followed by fetal growth restriction	(Gicquel *et al*., 2005) (Weksberg *et al*., 1993) (Steenman *et al*., 1994) (Constancia *et al*., 2002)
PLAGL1 (Pleiomorphic adenoma gene-like 1)	Apoptosis; Cell cycle regulation	Transient neonatal diabetes mellitus; IUGR (decreased expression)	IUGR; Altered bone formation; Increased neonatal lethality	(Varrault *et al*., 2006) (Diplas *et al*., 2009) (McMinn *et al*., 2006)
Maternal allele expressed				
PHLDA2 (Pleckstrin homology-like domain, family A, member 2)	Regulation of fetal and placental growth	IUGR (increased expression)	Placental overgrowth	(Frank *et al*., 2002) (Diplas *et al*., 2009) (McMinn *et al*., 2006)
H19 (Imprinted maternally expressed transcript)	Non-coding RNA	Silver Russell syndrome; Preeclampsia	Increased placental weight; Fetal overgrowth	(Gicquel *et al*., 2005) (Yu *et al*., 2009) (Leighton *et al*., 1995)
MEG3 (Maternally expressed gene 3)	Non-coding RNA	IUGR (decreased expression)	Fetal and postnatal growth reduction	(Schuster-Gossler *et al*., 1996) (McMinn *et al*., 2006)

2.2.3.2. Large-scale profiling of methylation in human placenta

The human placental DNA methylation profile is unique to the tissue and differs considerably from other somatic tissues (Christensen *et al*., 2009; Rakyan *et al*., 2008). The overall methylation in placenta is significantly lower compared to other tissues (2.5–3.5% versus 3.5–5%) and changes through the course of pregnancy (Fuke *et al*., 2004; Gama-Sosa *et al*., 1983). Novakovic *et al*. identified large-scale differences in DNA methylation levels between first, second, and third trimester placentas, with an increase in average methylation degree towards term pregnancy (Novakovic *et al*., 2011). Notably, imprinted alleles tend to have higher expression in first trimester placenta (Lambertini *et al*., 2008; Pozharny *et al*., 2010). Increased methylation of placental DNA appears to coincide with higher inter-individual variation of methylation (Novakovic *et al*., 2011). Interestingly, hypomethylation of placental DNA has been detected in repetitive DNA elements (human endogenous retroviruses) and in the inactivated X chromosome, although these sequence regions are silenced in other tissues (Cotton *et al*., 2009; Reiss *et al*., 2007). Also, human placenta shows inter-individual variation in DNA methylation (epipolymorphisms) (Yuen *et al*., 2009). More variable placental imprinting in early pregnancy may be explained by plasticity of early gene regulation, while the inter-individual variation in term placenta may be a response to environmental and stochastic exposures.

Besides studies of genome-wide DNA methylation in normal human placenta, the methylation patterns of diseased placenta are also gaining more attention. For instance, the hypomethylation of a collection of 34 CpG sites was recently described in placental samples of early-onset preeclampsia (Yuen *et al*., 2010). Distinctive patterns of DNA methylation were also found in placentas of IUGR (intrauterine growth restriction) and SGA pregnancies (small for gestational age) (Banister *et al*., 2011). Thus, DNA methylation in human placenta functions as a marker for the intrauterine environment, and can play a critical functional role in fetal development. Moreover, recent genome-scale methylation studies have taken advantage of the fact that about 3–6% of total free DNA in maternal plasma originates from placenta, and used the maternal blood to study placenta-specific methylation patterns to find biomarkers of pregnancy complications. For instance, mapping placenta-specific methylation of chromosomes 13, 18, 21, X and Y can be used as non-invasive diagnostic method for detecting trisomies of these chromosomes (Chim *et al*., 2008; Papageorgiou *et al*., 2009; Papageorgiou *et al*., 2011; Tsui *et al*., 2010).

3. AIMS OF THE STUDY

The general aim of the current thesis was to (a) compare transcriptional and epigenetic regulation of placental gene expression in normal and complicated pregnancies and (b) to identify genes with altered transcription and function in cases of pregnancy complications. I used both candidate gene-based and hypothesis-free approaches.

Based on the described state-of-art, the study had the following aims:

1. To conduct in-depth analysis of the expression of placenta-specific chorionic gonadotropin (HCG) beta subunit coding *CGB* genes:
   - Comparison of *CGB* gene expression in normal pregnancies and pregnancy complications (recurrent miscarriage, ectopic and molar pregnancy)
   - Investigation of epigenetic mis-regulation of the most actively transcribed *HCGβ* genes (*CGB8* and *CGB5*) in the pathogenesis of recurrent miscarriage

2. To perform the first transcriptome profiling of human placental gene expression in progression from early to mid-pregnancy (gestational weeks 5 to 18)
   - Identification of genes specifically upregulated in mid-gestation placenta;
   - Investigation the novel hypothesis that normal course of late pregnancy may be affected when genes characteristic to mid-gestation placenta remain highly expressed until term
   - Verification of protein expression of top-ranking candidate genes in pregnancy complications, and evaluation of their applicability as novel biomarkers.

4. RESULTS

4.1. Part I – targeted investigation of the transcription of the placenta-specific human chorionic gonadotropin beta genes (Ref. I, II, III)

4.1.1. Placental transcription of *HCGβ*-coding genes in normal and complicated pregnancies

Since the final production of human chorionic gonadotropin (HCG) is determined by the transcription of the four *HCGβ*-coding genes, their detailed mRNA transcription profile during normal and disturbed pregnancies was addressed. This study was carried out under the leadership of Dr. Kristiina Rull (Tartu University Hospital, Women's Clinic, Tartu, Estonia). Trophoblast samples were collected from the first, second and third trimesters of uncomplicated pregnancies as well as first trimester pregnancies with complications (recurrent miscarriage, ectopic and molar pregnancy; details in **Table 4**) and analysed jointly for *HCGβ* expression.

Table 4. Samples used in RT-qPCR expression analysis of *HCGβ*-coding genes.

Study group	No. of individuals	Gestational age (weeks)
1st trimester, normal	10	4–12
2nd trimester, normal*	8	17–21
3rd trimester, normal	12	38–42
1st trimester, ectopic pregnancy	8	6–14
1st trimester, recurrent miscarriage**	11	6–17
1st trimester, molar pregnancy***	2	9–10

*Therapeutic abortion during 2nd trimester due to medical risks of pregnancy; no fetal anomalies were detected.
**Patients had had ≥2 spontaneous abortions before the case.
***Molar pregnancy is characterised by a reduced or even lack of embryonic development and excessive trophoblast proliferation.

To identify the total expression of *HCGβ*-coding genes, sensitive RT-qPCR quantification approach was used for selected target genes and housekeeping genes (*GAPTH*, glyceraldehyde phosphate dehydrogenase; *HPRT1*, hypoxanthine phosphoribosyltransferase 1). Due to high sequence similarity of *HCGβ* coding genes, transcription levels were assessed using a shared primer set that amplified all *HCGβ*-coding genes (**Materials and Methods in Ref. II**).

We found that the expression of *HCGβ*-coding genes shows significant variation over the course of human pregnancy. Total *HCGβ* transcription is highest in the first trimester compared to the second (P=0.01) and third trimester of gestation (P=0.11). Importantly, first trimester complications associated to significant changes in *HCGβ* transcription. In particular, placentas from ectopic pregnancies (EP) showed higher levels of *HCGβ* transcription (P=0.06) and placentas from recurrent miscarriages (RM) had lower levels of *HCGβ* (P=0.03). However, the latter group of complications is characterised by considerable rate of inter-individual variability (**Figure 5**). Our findings support the outcome from previous studies that used semi-quantitative expression analyses of *HCGβ*-coding genes in normal and complicated pregnancies (Miller-Lindholm *et al*., 1997; Rull and Laan, 2005). We also reported similar findings between placental expression profile of these genes and protein levels of HCG in blood serum during normal and complicated pregnancies (Berkowitz *et al*., 1989; Hay, 1988; Letterie and Hibbert, 2000).

Main conclusions and implications of this study:

1. Besides showing considerable inter-individual variation of HCG hormone levels, the study demonstrated inter-individual differences in total transcription levels of *CGB* genes between pregnancies of same gestational age or clinical group.
2. Uncomplicated pregnancies are characterized by the highest total expression of *CGB* genes in the first trimester, followed by a sharp decrease in expression in the second trimester and modest increase in term placenta.
3. Significantly lower expression of total *HCGβ*-coding mRNA in recurrent miscarriage explains the decreased HCG hormone levels in RM and potentially refers to aberrant transcription of *CGB* genes. High expression of *HCGβ*-coding genes but low hormone level in EP complications may result from defects in hormone assembly.

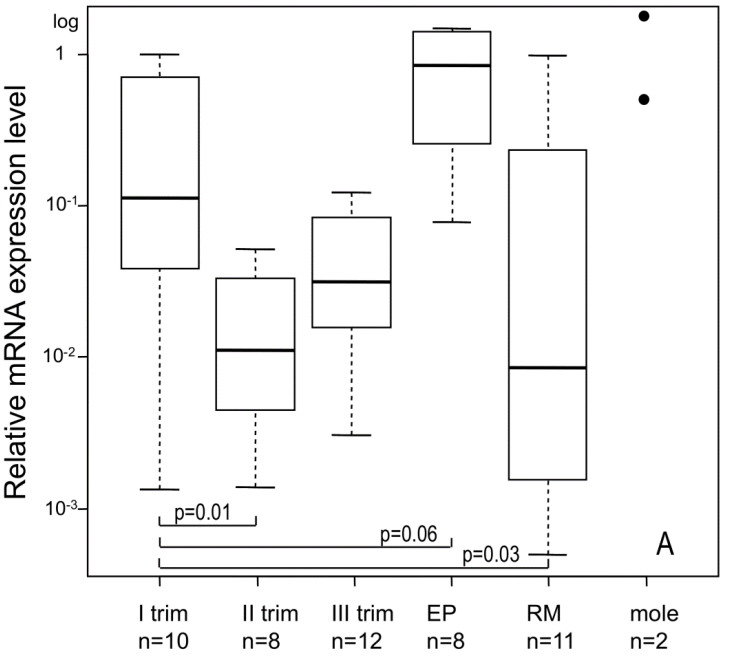

Figure 5. Relative expression level of total *CGB* transcripts in placenta during the first, second and third trimester of normal pregnancy, in cases of recurrent miscarriage (RM), ectopic pregnancy (EP) and molar pregnancy (**Ref. II**).

4.1.2. Epigenetic regulation of *HCGβ*-coding genes

Significantly lower expression level of *HCGβ*-genes in RM placentas may refer to transcriptional or post-transcriptional alterations that induce silencing of the *HCGβ*-genes. Post-transcriptional silencing mechanisms destroy the synthesized mRNA (e.g. RNA interference) or block its translation (Hammond *et al*., 2001). Transcriptional silencing involves transcriptional repressor proteins and epigenetic mechanisms such as DNA methylation and chromatin modifications (Attwood *et al*., 2002).

In the second study (**Ref. III**) I tested the hypothesis that the placental expression of *HCGβ*-coding genes is imprinted, and the decrease in total *HCGβ* level in RM cases refers to aberrant DNA methylation. The hypothesis originated from the observation that the genomic region of *HCGβ*-coding genes has several features common to other placental imprinted genes (Bressan *et al*., 2009). First, the *LHB/CGB* region has a high GC nucleotide content (≥55%), high repeat content, and abundance of CpG islands (Hallast *et al*., 2005) that are likely targets of DNA methylation. Second, imprinted genes often cluster in common genomic regions (Verona *et al*., 2003) and the *HCGβ* region co-occurs

with the imprinted gene *PEG3* (paternally expressed gene 3, 19q13.4) (Hiby *et al*., 2001).

I chose to investigate the expression of *CGB5* and *CGB8* genes, as they contribute up to 82% of total *CGB* transcription pool (Miller-Lindholm *et al*., 1997; Rull and Laan, 2005). Transcriptional changes of these genes therefore cause the greatest changes in HCG hormone level.

4.1.2.1. Successful pregnancy requires biparental expression of CGB5 and CGB8

My first objective was to determine the parental origin of placental transcription of *CGB5* and *CGB8* alleles with family-based (mother/father/placenta) genotyping of single nucleotide polymorphisms (SNPs). Both placental and parental blood samples from first and third trimester uncomplicated pregnancies and from cases of first trimester RM complications were collected (Dr. Kristiina Rull, Tartu University Hospital Women's Clinic). A total of 23 mother-offspring duos and nine mother-father-offspring trios were analysed (**Figure 6A**).

Three SNP positions located in either *CGB5* (rs710899; ss105107003; rs12610392) or *CGB8* (rs34212754; rs13345685; rs35930240) gene 5'UTR regions were genotyped using long-range and gene-specific PCR and resequencing methods (**Figure 1B and Figure 2A** in **Ref. III**). The placental samples were considered genetically informative if they were heterozygous for at least one SNP out of three, such that at least one parent carried a homozygous genotype for the same SNP (**Figure 1D in Ref. III**). Using this approach, I identified 14 informative placental samples for *CGB8* and nine informative placentas for the *CGB5* gene (**Figure 6A**; **Table 1 and Table 2 in Ref. III**).

Next, the parental origin of mRNA sequences of informative *CGB8* and *CGB5* cases was detected using cloning and re-sequencing methods (**Figure 6B**). Most analysed placentas (87%, 20 out of 23) showed biparental expression of *CGB5* and *CGB8* genes in normal first and third trimester samples as well as in RM cases (**Table 1 and Table 2 in Ref. III**). Interestingly, I found monoallelic maternal expression of *CGB5* in three placentas, including two RM cases and one elective termination of first trimester pregnancy (**Table 1 in Ref. III**). Due to the small sample size and limited number of informative cases, the association between RM and monoallelic *CGB5* expression is not statistically significant (Fisher's exact test P=0.23, 9 informative samples).

The one healthy sample with monoallelic expression (ETP26) originates from an exceptionally early elective termination of pregnancy (week 4) classified as a normal placenta. However one can speculate on the possible complications that could have occurred at the later stages of this pregnancy. When excluding this sample as an outlier, we gain increased significance of RM association (P=0.11, 8 samples).

In conclusion, this result rejected the hypothesis that *CGB* genes are normally imprinted in human placenta, since biallelic expression was detected in most of the genetically informative cases for the parental origin of alleles.

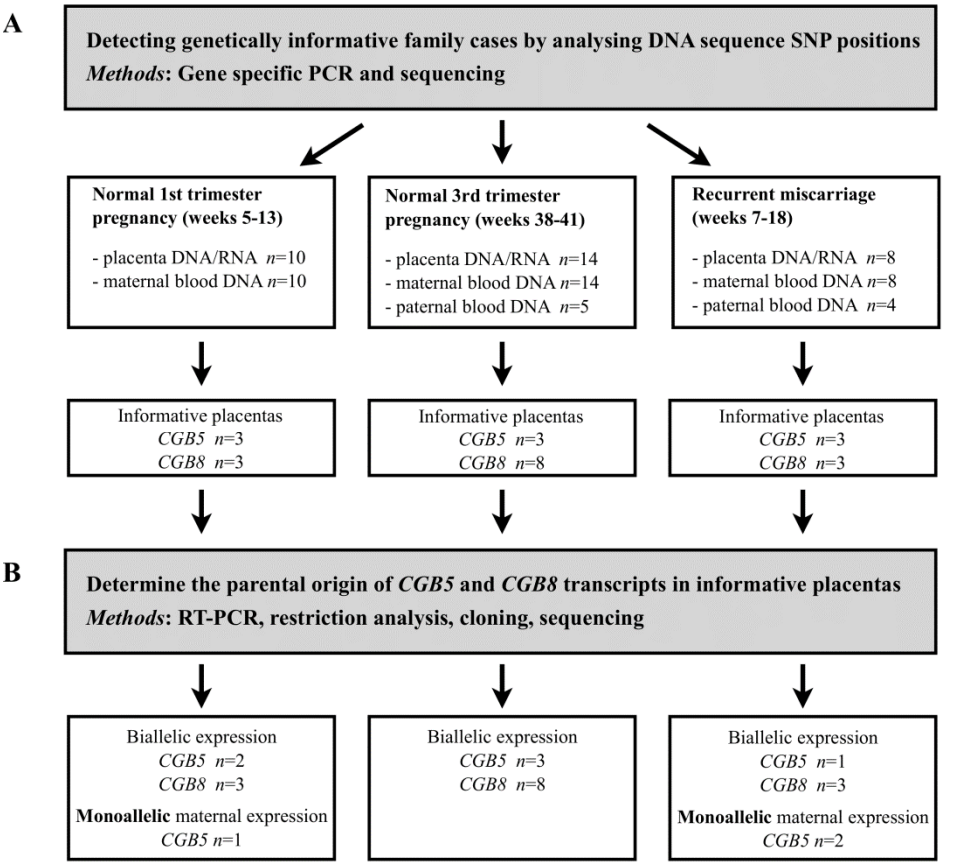

Figure 6. Overview of analysed samples and methods used for detecting parental of origin of placentally expressed *CGB5* and *CGB8* alleles. **A.** Among analysed samples, nine placentas were confirmed as informative for the *CGB5* gene and 14 were informative for the *CGB8* gene. **B.** Most informative placentas showed biparental expression for both *CGB5* and *CGB8* gene, except for two RM cases and one uncomplicated 1st trimester placenta where only maternally expressed *CGB5* alleles were detected. Recurrent miscarriage; patients had experienced ≥2 spontaneous abortions before the case.

4.1.2.2. Uniparentally expressed CGB5 promoter is hemimethylated in placenta

Though the production of *HCGβ*-subunit of HCG is guaranteed by biparental expression of *CGB5* and *CGB8* for the most of normal pregnancies, I found that the expression of paternally inherited allele of *CGB5* was inhibited in some placentas. Parent-of-origin specific silencing of one gene allele that is normally expressed biallelically may refer to allele-specific promoter methylation (Bartolomei and Ferguson-Smith, 2011).

To gain insight into the methylation of 13 CpG sites in the promoter of *CGB5*, I used the methylation-sensitive amplification (MS-PCR) of sodium bisulfite treated genomic DNA, followed by clonal sequencing (details in **Ref. III, Subjects and methods, Figure 4A**). Three groups of samples were studied: (i) placentas of uniparentally expressed *CGB5* (*n*=3), (ii) normal term placentas of biparentally expressed *CGB5* (*n*=2) and (iii) parental peripheral blood (*n*=7).

I found that all parental DNA samples of both complicated and uncomplicated pregnancies showed nearly complete methylation of *CGB5* promoter, while the promoters were unmethylated in placentas of uncomplicated pregnancies. In contrast, three placental samples with only maternal *CGB5* expression revealed both methylated and unmethylated promoter variants (**Figure 3 and Figure 4B in Ref. III**). This novel methylation allelic polymorphism (MAP) in *CGB5* promoter correlates with the identified expression patterns of *CGB5*. Monoallelic maternal expression was accompanied with DNA hemimethylation in *CGB5* promoter whereas biallelic expression was observed in all unmethylated cases. Unfortunately the parental origin of the methylated and unmethylated alleles was not detectable due to absence of marker SNPs in the analysed region after bisulfite treatment.

Methylation of transcription factor binding sites is a potential mechanism of parent-specific transcriptional silencing of *CGB5* expression. One of the analysed CpG sites is located 198 bp upstream of the *CGB5* transcription start site, within a functional Sp1 transcription factor binding site (Johnson and Jameson, 1999). The site was hemimethylated in all three cases with uniparentally expressed *CGB5* (**Figure 4B in Ref. III**). This observation suggests that methylation of CpG site disrupts transcription factor binding and alters the transcription of downstream *CGB5* mRNA.

In addition to the above, Turner syndrome (45 X monosomy) was diagnosed in one of the three RM cases with MAP and monoallelic CGB5 expression (**Supplemental Figure 1 in Ref. III**).

Main conclusions and implications of this study:

1. Biparental expression of the *CGB5* and *CGB8* genes is required for normal outcome of pregnancy.
2. Monoallelic maternal expression and promoter hemimethylation of *CGB5* gene is associated with recurrent miscarriage.
3. *CGB5* may be a novel epipolymorphic gene with occasional gain of paternal imprinting (GOI).

4.2. Part II – genome-wide study: mid-gestation placental gene expression and link to pregnancy complications (Ref. IV)

Despite the great importance of placenta in mediating the rapid physiological changes in pregnancy, data on the temporal dynamics of human placental gene expression are limited. In this part of the thesis I studied genome-wide placental gene expression during three trimesters of uncomplicated gestation. The goal of this study was to detect mid-gestation specific genes, and explore the expression of mid-gestation specific genes and proteins in normal pregnancies and pregnancy complications.

4.2.1. Genome-wide transcriptome profiling of early and mid-gestation placentas

To describe the transcriptional changes in placenta during the first and second trimester of human pregnancy, ten placental samples from gestational weeks 5–18 were analysed on GeneChip (Affymetrix) expression arrays (**Figure 7A**). Subsequent gestational age-dependent quantitative microarray analysis detected 154 differentially expressed genes (ANOVA; FDR corrected $P<0.1$), including 105 genes with gradually increasing and 49 genes with decreasing transcript levels (**Figure 1** and **Table S2 in Ref. IV**). This analysis revealed genes with dynamic changes in gene expression during three months of early pregnancy. Alternatively, microarray data was subjected to differential expression analysis between placental samples grouped as 'early' ($n=6$) or 'mid-gestation' ($n=4$). The second analysis identified 205 genes with significantly higher expression and 24 genes with significantly lower expression in mid-pregnancy (*t*-test; FDR corrected $P<0.1$) (**Figure S3** and **Table S4 in Ref. IV**). The majority of gradually up-regulated genes identified in the first analysis (n=63, 60%) were also found in the second analysis, suggesting that both statistical strategies identified biologically relevant genes.

The most significantly up-regulated genes during first and second trimester of gestation according to the quantitative analysis of microarray data are listed in **Table 5** (supplemental information in **Figure 2** and **Table S2 in Ref. IV**). Many of my findings are related to fetal development. For example *FST* (follistatin), *BMP5* (bone morphogenetic protein 5), *STC1* (stanniocalcin 1) and *CDH11* (cadherin 11) participate in bone and skeleton development (Cheng *et al*., 1998; Gajos-Michniewicz *et al*., 2010; Yeung *et al*., 2012; Zoricic *et al*., 2003). *NRCAM* (neuronal cell adhesion molecule) and *GATM* (glycine amidinotransferase) genes are involved in the development of the nervous system (Braissant *et al*., 2005; Grumet, 1991). *PLAGL1* (Pleiomorphic adenoma gene-like 1) regulates cardiac and pancreas morphogenesis (Du *et al*., 2011; Yuasa *et al*., 2010) (**Table 5**; **Table S9** in **Ref. IV**).

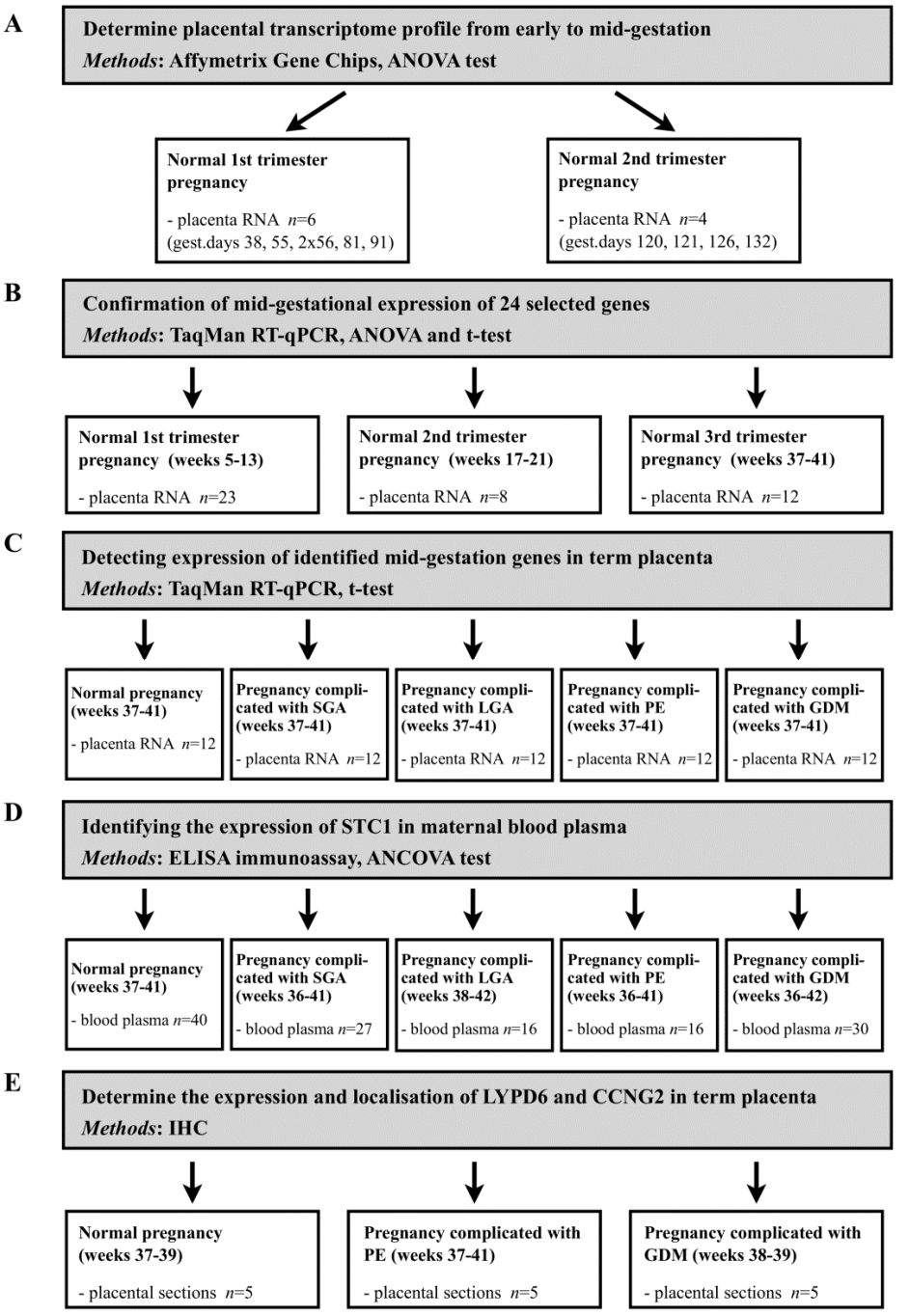

Figure 7. Overview of analysed samples and methods used for identification of mid-gestational specific genes in placenta (**A**, **B**) and detecting the gene and protein expression of mid-pregnancy markers in pregnancy complications (**C**, **D**, **E**). PE, pree-clampsia; GDM, gestational diabetes mellitus; SGA, small for gestational age; LGA, large for gestational age.

Table 5. Mid-gestation marker genes with significantly increased expression in placenta identified by Affymetrix® GeneChips and RT-qPCR validation. ANOVA test was used for both microarray and RT-qPCR statistical analyses.

Gene name	Full name	Affymetrix array[a]		RT-qPCR[b]		Biological function[c]
		P-val[d]	FC[e]	*P*-val[d]	FC[f]	
FST	Follistatin	0.007	7.2	3×10^{-12}	2.7	Inhibition of FSH release, folliculogenesis, bone mineralization, muscle growth
ITGBL1	Integrin, beta-like 1	0.037	6.1	10^{-9}	3	Cell adhesion
LYPD6	LY6/PLAUR domain containing 6	0.037	3.5	5×10^{-11}	3	Transcriptional regulation; tumor suppressor
NR3C1	Nuclear receptor subfamily 3, group C, member 1	0.037	2.7	10^{-7}	1.6	Transcriptional regulation, chromatin remodelling, cellular proliferation and differentiation, inflammatory response
NRCAM	Neuronal cell adhesion molecule	0.037	9.1	4×10^{-8}	3.1	Nervous system development, cell adhesion
SLC16A10	Solute carrier family 16, member 10	0.048	7.1	4×10^{-9}	3.1	Aromatic amino acids transport, thyroid hormone transport
GPR183	G protein-coupled receptor 183	0.049	7	7×10^{-8}	2.8	Humoral immunity
NEDD9	Neural precursor cell expressed, developmentally down-regulated 9	0.052	4.9	3×10^{-6}	1.7	Tyrosine-kinase-based signalling related to cell adhesion, proliferation
CCNG2	Cyclin G2	0.062	2.4	2×10^{-6}	1.4	Negative regulation of cell cycle, adipogenesis; proliferation and differentiation of uterine cells in implantation and decidualization

Gene name	Full name	Affymetrix array[a]		RT-qPCR[b]		Biological function[c]
		P-val[d]	FC[e]	*P*-val[d]	FC[f]	
BMP5	Bone morphogenetic protein 5	0.065	13.4	7×10^{-11}	4.2	Bone, cartilage and limb development, skeletal growth
ZFP36L1	Zinc finger protein 36, C3H type-like 1	0.065	5.6	7×10^{-4}	2.3	Regulation of translation; RNA metabolic process; regulation of mRNA stability and decay
PLAGL1	Pleiomorphic adenoma gene-like 1	0.068	2.7	10^{-9}	3	Transcription factor, apoptosis, embryonic development and growth; cell cycle arrest, cardiac morphogenesis, development of pancreas
GATM	Glycine amidinotransferase	0.079	7.8	7×10^{-9}	3.1	Creatine biosynthesis, kidney function, nervous system development
STC1	Stanniocalcin 1	0.083	6.9	2×10^{-8}	2.2	Renal and intestinal Ca2+/P homeostasis, bone and muscle development, kidney function, gestational and nursing state regulator
MEG3	Maternally expressed 3	0.091	10.6	2×10^{-10}	6.4	Non-coding RNA, negative regulation of cell proliferation, embryonic development, tumor suppressor
CDH11	Cadherin 11	0.098	3.1	0.0016	1.7	Cell adhesion, bone formation, growth, maintenance and morphology, tumor suppressor

[a] First trimester samples, n=6 (weeks 5–13); second trimester samples, n=4 (weeks 17–19).
[b] First trimester samples, n=23 (including discovery samples, weeks 5–13); second trimester samples, n=8 (including discovery samples, weeks 17–21).
[c] References are listed in **Ref. IV Table S9**.
[d] ANOVA test *P*-values are FDR corrected.
[e] Microarray fold change (FC) was calculated from the mean of 5th and 18th week placental samples.
[f] RT-qPCR fold change was calculated as the difference of mean relative expression values of first (n=23) and second trimester samples (n=8).

4.2.2. Functional enrichment analysis of differentially expressed genes

The functional enrichment analysis of significantly differentially expressed genes using g:Profiler software (Reimand *et al*., 2011) revealed more than 200 statistically over-represented Gene Ontology (GO) categories and pathways for genes with increased placental expression towards mid-pregnancy (**Table S6 in Ref. IV**). The transcriptional patterns in the evolving placenta are characterized by biological processes such as anatomical structure development, growth, cell communication and adhesion, cell surface receptor signalling and cellular response to stimulus (hypergeometric test, FDR $P<10^{-3}$). In addition, processes related to pregnancy development and maintenance such as blood vessel development, VEGF receptor signalling, and gonadotropin secretion were observed (FDR $P<10^{-3}$). Considerably fewer enriched GO biological processes were detected for genes with decreasing expression. The most significant down-regulated process was transcription from RNA polymerase III promoter (FDR $P=1.7x10^{-4}$). These functional enrichments provide support to the identified placental genes.

4.2.3. RT-qPCR assays identify multiple classes of mid-gestation specific genes

A total of 24 genes were prioritized from the microarray analysis for further experimental confirmation by quantitative RT-qPCR analysis, using an extended set of first (n=23), second (n=8) and third trimester (n=12) placentas (details provided in **Materials and Methods in Ref IV**) (**Figure 7B; Figure 2** and **Table S2** in **Ref IV**). Third trimester samples of uncomplicated pregnancies were included to determine the expressional dynamics of mid-gestation genes throughout pregnancy. The names and functions of prioritized genes are shown in **Table S3** in **Ref. IV**.

The validation experiment largely confirmed findings of the microarray analysis. As a result, several mid-gestation specific placental genes were confirmed. I found 16 genes (*FST*, *MEG3*, *PLAGL1, ITGBL1, BMP5, STC1, GPR183, LYPD6, CDH11*, *SLC16A10, NRCAM*, *GATM, CCNG2*, *NEDD9, NR3C1, ZFP36L1*) that showed significant increase in expression throughout the first and second trimester of pregnancy (ANOVA and t-test, FDR $P<0.005$; **Table 5**; **Figure 3**, **Figure 4**, **Table 2** and **Table S7 in Ref. IV**). The analysis revealed three types of expression patterns. First, ten genes showed low expression at first trimester, a clear expressional peak at mid-gestation followed by up to 4.2-fold decrease in transcript levels at term (*BMP5*, *CCNG2*, *CDH11*, *FST*, *GATM*, *GPR183*, *ITGBL1*, *PLAGL1*, *SLC16A10*, *STC1*; all FDR $P<0.05$, t-test; **Figure 4A** and **Table S7** in **Ref. IV***)*. Second, four genes (*LYPD6*, *MEG3*, *NRCAM*, *ZFP36L1*) were significantly upregulated in second trimester samples and remained highly transcribed until term (**Figure 4B** in **Ref. IV**). Third, two

genes (*NEDD9*, *NR3C1*) were increasingly upregulated throughout gestation (**Figure 4C** in **Ref. IV**). These gene groups are likely to represent different regulatory programs and developmental functions.

4.2.4. Altered expression of mid-gestational genes in late pregnancy complications

Normal course of late pregnancy may be affected when the genes characteristic to mid-gestation placental transcriptome remain highly expressed until term. The next aim of this study was to test the hypothesis that aberrant third trimester gene expression of mid-gestation placental genes contributes to fetal growth, or the development of maternal pregnancy complications at term.

Placental samples from normal third trimester pregnancies (n=12) were compared to samples from complicated pregnancies of maternal gestational diabetes (GDM, n=12), preeclampsia (PE, n=12), or disturbed fetal growth (SGA, small for gestational age, n=12; LGA, large for gestational age, n=12) (**Figure 7C**). Study groups are described in detail in **Table 1A** of **Ref. IV**.

Seven of the 16 quantified (RT-qPCR) mid-gestation genes were transcribed significantly higher in placental samples of pregnancy complications compared to the control group (**Table 6**; **Table 3** in **Ref IV**). In addition to two-group statistical tests, we used analysis of covariance (ANCOVA) tests to account for confounding factors such as gestational age and mother's age that potentially influence gene expression. Three genes *STC1*, *LYPD6* and *CCNG2* were found to have the strongest association with pregnancy complications. *STC1* showed increased placental expression in all studied patient groups, while higher expression of *LYPD6* and *CCNG2* associated to PE and GD complications. *LYPD6* also associated to smaller newborns (SGA). Details of these results are shown in **Table 6** of the thesis as well as **Table 3** in **Ref IV**.

Table 6. Mid-gestational marker genes with increased mRNA expression levels in placentas of term pregnancy complications compared to normal third trimester placentas.

	Maternal preeclampsia		Maternal gestational diabetes mellitus		Baby small for gestational age		Baby large for gestational age	
Gene ID	t-test	ANCOVA	t-test	ANCOVA	t-test	ANCOVA	t-test	ANCOVA
STC1	**$<10^{-3}$**	NS	0.021	NS	**$<10^{-3}$**	**$<10^{-3}$**	$<10^{-2}$	NS
LYPD6	**$<10^{-2}$**	NS	**$<10^{-4}$**	0.026	0.014	**$<10^{-2}$**	$<10^{-2}$	NS
CCNG2	**$<10^{-3}$**	**$<10^{-2}$**	**$<10^{-3}$**	**$<10^{-3}$**	NS	NS	0.027	NS
GATM	0.025	NS	0.024	0.041	0.022	0.043	0.019	NS
GPR183	NS	NS	NS	NS	0.025	NS	0.029	NS
MEG3	NS	NS	0.012	NS	NS	NS	NS	NS
CDH11	NS	NS	0.026	NS	NS	NS	NS	NS

Four further genes did not pass the statistical thresholds for group comparisons (*t*-test, FDR *P*>0.05). However, a weak correlation of increased expression and pregnancy complications was observed (**Table 6**). For example, higher transcription of *GATM* was detected in all complication groups compared to normal term placentas, *GPR183* showed increased expression in cases of affected fetal growth, and expression of *MEG3* and *CDH11* were increased in placentas complicated of GD (**Table 6**; **Table 3** in **Ref IV**).

Seven mid-gestation marker genes with at least one statistically significant association to pregnancy complications were found. Numbers indicate *P*-values. Bold letters highlight significant results after FDR correction. Analysis of covariance (ANCOVA) was used for seven genes with significant values from t-test. ANCOVA tests were adjusted by gestational age, placenta weight, infant gender and type of delivery. GD tests were additionally adjusted by infant weight and maternal age; PE tests with infant weight. ANCOVA FDR considered 7 genes.

4.2.5. Verification of protein expression

The three genes (*STC1*, *CCNG2*, *LYPD6*) with the strongest statistical significance of differential expression in complicated term placentas were selected for the analysis of protein expression, either in maternal plasma by ELISA (STC1), or in placental tissue samples by immunohistochemistry (IHC) (CCNG2, LYPD6).

4.2.5.1. STC1 maternal plasma protein level is elevated in pregnancy complications

The *STC1* gene, characterized by sharp upregulation of placental gene expression in mid-gestation, encodes as a soluble glycoprotein hormone Stanniocalcin 1 (STC1) (Jellinek *et al*., 2000). In this study, STC1 concentrations were measured in blood plasma collected on the day of delivery from women of normal term pregnancies (*n*=40) as well as patients from pregnancies complicated with PE (*n*=16), GD (*n*=30) or affected fetal growth (SGA, *n*=18; LGA, *n*=16) (**Figure 7D**; **Table 1B** in **Ref. IV**). I aimed to test whether the increased expression of *STC1* in pregnancy complications correlates with higher maternal circulating STC1 levels.

Significantly elevated plasma STC1 levels were detected in SGA, PE and GD groups of pregnancy complications (median concentrations >650 pg/ml) compared to healthy controls (median concentration 418 pg/ml) (ANCOVA, *P*<0.05, **Figure 5A** in **Ref IV**). Moreover, when grouping PE cases according to newborn birth weight, increase of maternal plasma concentrations of STC1 was more pronounced in the PE-SGA group (median 731 pg/ml; ANCOVA, *P*=0.00048; **Figure 5B** in **Ref IV**).

4.2.5.2. Expression and localisation of LYPD6 and CCNG2 proteins in placental tissue of normal term and complicated pregnancies

The expression of cytoplasmic proteins LYPD6 (Zhang *et al*., 2010) and CCNG2 (Bennin *et al*., 2002) was measured concurrently using placental paraffin sections sampled from five control, five PE and five GDM pregnancies at term (details in **Materials and Methods**, **Table S1** and **Text S3** in **Ref. IV**).

Both LYPD6 and CCNG2 showed enhanced immunostaining in placental samples of PE and GDM complications compared to controls. This result confirms our previous observations and correlates with RT-qPCR results of increased transcription of *LYPD6* and *CCNG2* in PE and GDM placentas (**Figure 8**; **Figure S5**, **Figure S6**, **Table 3** in **Ref. IV**). Specifically, LYPD6 staining was detected in villous syncytiotrophoblast of all studied samples, as well as in villous Hoffbauer cells, fibroblasts and endothelial cells of villous vessels (**Figure 8**; **Figure S5** in **Ref. IV**). Similarly, I found the CCNG2 protein to be specific to villous stromal Hoffbauer cells and fibroblasts, while only weak staining was seen in syncytiotrophoblast and endothelial cells of vessel walls (**Figure 8**; **Figure S6** in **Ref. IV**).

Main conclusions and implications of this study:

1. This is the first study to report quantitative profiling of placental transcriptome dynamics over the first and second trimester of pregnancy (weeks 5 to 18).
2. Ten genes with peaking expression in mid-gestation placenta were validated with RT-qPCR in the comparison of early and term pregnancies.
3. The study demonstrated the importance of fine-scale tuning of dynamic transcriptional regulation during gestation: several mid-gestation genes showed aberrant expression in PE, GDM and SGA placentas at term compared to controls.
4. The circulating STC1 protein has a direct potential as a prognostic, non-invasive maternal serum biomarker for determining patients with complex pathologies in which preeclampsia is accompanied with restricted fetal growth.
5. As an additional novel finding, I found that most of the identified genes with peaking expression in mid-gestation placenta have also been implicated in adult complex diseases (**Table S9** in **Ref. IV**). This observation supports the recent discussion regarding the role of placenta in developmental programming (McKay, 2011) and *in utero* origin of adult disease (Barker *et al*., 2002).

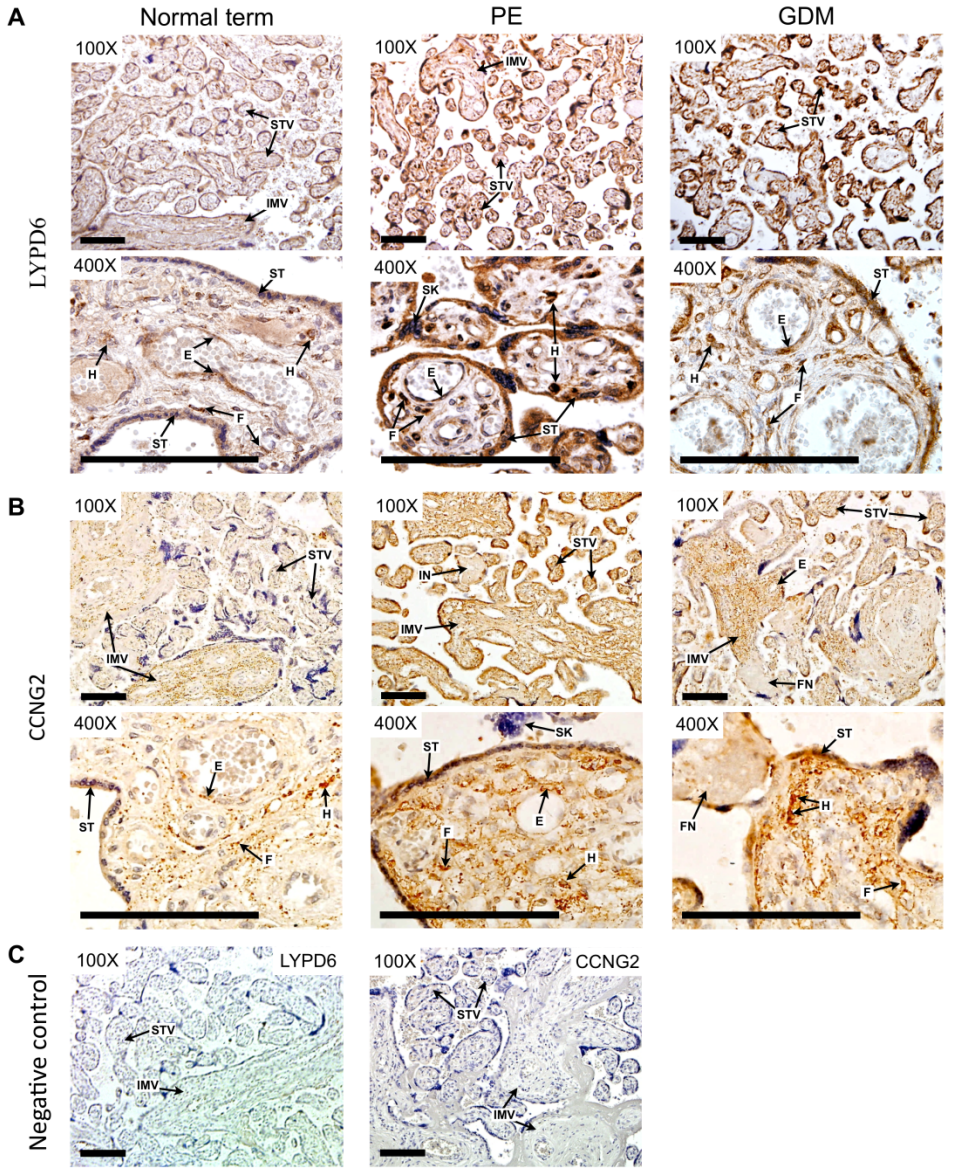

Figure 8. Immunostaining of LYPD6 and CCNG2 proteins in placental sections from uncomplicated control, preeclampsia (PE) and gestational diabetes mellitus (GDM) pregnancies at term. A. Strong LYPD6 antibody staining was found in cytoplasm and nucleus of villous stromal Hoffbauer cells (H), fibroblasts (F) and endothelial cells (E) of villous vessels. Additionally, diffuse cytoplasm staining of LYPD6 was detected in syncytiotrophoblast (ST). **B.** CCNG2 antibody showed fine granular cytoplasm staining of villous Hoffbauer (H) and fibroblast (F) cells, and weak staining of syncytiotrophoblast (ST) and endothelial cells (E) of villous vessels. No localisation differences in LYPD6 or CCNG2 antibody staining were detected between control, PE and GDM samples, although higher staining intensity was observed in PE and GDM placentas compared to controls. **C.** Negative control (NC) staining without primary antibody. STV, small terminal villi; IMV, intermediate villi; SK, syncytial knot; IN, infarction lesion; FN, fibrinoid necrosis. Microscope magnifications x100 and x400 were used; scale bar, 50 μm.

5. DISCUSSION

5.1. Challenges of placental gene expression and epigenetic studies

The human placenta is gaining increasing scientific attention as its role in physiology and disease is further acknowledged. Placenta holds a critical structural and functional role of mediating fetal development and maternal adaption during pregnancy. It also acts as a central interface in fetal and maternal programming. Maternal and fetal pregnancy-related conditions involving placenta may contribute to the health of mother and child later in their lives. Therefore, understanding the function of placenta at the molecular level is crucial for health and well-being. However, there are a number of limitations that restrict human placental gene expression studies.

First, placental studies on human tissue are complicated due to restricted collection of ethically sensitive biomaterial, resulting in reduced sample sizes and small power of reported statistical tests. Several placental studies published to date have used samples from either a) early stages of pregnancy originating from elective termination of pregnancy either due to personal reasons (elective termination of pregnancy) or medical conditions (spontaneous abortion); or b) placental tissue sampled after delivery of baby at term. Collecting mid-term placental samples is especially difficult due to clinical and ethical restrictions. Consequently, only few current studies have explored the molecular biology of mid-gestation human placenta (Mikheev *et al*., 2008; Winn *et al*., 2007). While the study of placental epigenetic and transcriptional regulation in this doctoral thesis is novel in its scope and findings, the relatively small number of analysed samples ultimately affects its conclusions. In particular, investigation of epigenetic imprinting and parent-of-origin of allelic expression requires extensive collection to accumulate a meaningful set of informative samples. In this doctoral thesis, I obtained and analysed a number of family duos (placental samples and maternal blood samples) and trios (placental samples, maternal and paternal blood samples). However, as the coding regions of *CGB* genes showed relatively little variability and corresponding SNPs were sparse, the small number of informative families limited the interpretation of results of the methylation study.

Second, human hemochorial placentation has been considered as the most invasive placentation process among mammals (Benirschke *et al*., 2006). Cytotrophoblast cells invade maternal *decidua basalis* through rapid proliferation, attaching the placenta to the mother's uterus (Boyd, 1970). Consequently, the interpretation of results from placental gene expression and epigenetic studies is complicated as the collected placental samples often contain a mixture of fetal and maternal cells. Moreover, placenta itself is heterogeneous and contains multiple types of cells such as syncytiotrophoblasts and cytotrophoblasts with their own physiological functions as well as transcriptional and epigenetic

signatures (Avila *et al*., 2010; Penaherrera *et al*., 2012). Such heterogeneity at the cellular level underlines the need to investigate transcriptional profiles of placental samples collected from different locations of the same tissue. Surprisingly however, levels of DNA methylation have been found to be consistent across different locations throughout term placenta (Avila *et al*., 2010; Ferreira *et al*., 2011; Non *et al*., 2012). While recent technological developments allow placental sampling and differentiation of cell layers from term placentas, it is not yet possible to achieve clear trophoblast differentiation of maternal or fetal cells from samples of early pregnancy. Therefore transcriptional analysis of early placental samples is more likely to reflect cells of the maternal-fetal interface rather than homogeneous placental cells.

Third, transcriptional analysis of placental tissue is complicated because no reference genes with stable expression across all three trimesters of pregnancy are known. In this doctoral work, the housekeeping genes *HPRT1* and *GAPDH* were used as references in RT-qPCR experiments. *HPRT1* has been shown previously to maintain a stable expression level during early pregnancy (Khan *et al*., 2010), as well as in comparisons of complicated placentas (GD, PE) and normal term placentas (Lanoix *et al*., 2012; Meller *et al*., 2005). The stability of *GAPDH* expression has been confirmed in normal term placentas (both labour and caesarean section) and placentas of fetal growth restriction (Murthi *et al*., 2008; Patel *et al*., 2002). However, differential expression of *GAPDH* has been detected between first and third trimester of normal human placentas (Patel *et al*., 2002), indicating that it may be an unfavourable reference gene for expression studies considering all three trimesters of pregnancy.

5.2. Modulation of placental gene expression by interindividual variation of methylation and pregnancy outcome

DNA methylation is a mitotically heritable but reversible regulatory mechanism that produces different tissues, cell types and phenotypic variability. Methylation depends of the individual's sex and age, but it is also stochastic and prone to changes induced by environmental stimuli (Grigoriu *et al*., 2011; Jaenisch and Bird, 2003; Novakovic *et al*., 2011). DNA methylation is a common mechanism of epigenetic silencing of gene expression and it plays an especially important role in placental transcription control (Bird, 2002; Diplas *et al*., 2009). Placental DNA methylation is required for normal fetal development and maternal adaption to pregnancy. Methylation follows a dynamic pattern to ensure fine-scale time-dependent tuning of placental gene expression.

In my doctoral thesis I investigated the DNA methylation patterns of *CGB5* and *CGB8* genes. I compared cases of uncomplicated pregnancies and recurrent miscarriages to explain our findings of significantly lower total *HCGβ*-coding gene expression in cases of RM. The initial hypothesis considered potential

imprinting of *CGB* genes in placenta and aberrant methylation in recurrent miscarriage. Candidate *CGB8* and *CGB5* genes were selected as they contribute together up to 82% of total pool of *HCGβ* transcripts (Miller-Lindholm *et al*., 1997; Rull and Laan, 2005). About 50% of RM cases are currently not linked to clinical causes (Jauniaux et al., 2000), suggesting a potential role of genetic determinants. In this study, I was able to take advantage of a unique selection of family duos and trios and determine parent of origin of expressed alleles in placenta. Differential methylation of *HCGβ*-genes has been previously observed in trophoblast and tumour cell lines (Campain *et al*., 1993) supporting the hypothesis that *HCGβ*-coding genes may be imprinted in placenta.

I detected biallelic expression of *CGB8* and *CGB5* genes in most of the analysed trophoblast samples, including both RM and normal cases, thus refuting the initial hypothesis of imprinted expression of *CGB* genes. However, I also observed imbalance of expressed alleles in a subset of analysed placentas: only maternally derived *CGB5* alleles were expressed in three of nine informative placentas (one ETP and two RM cases) and the promoter region of *CGB5* was hemimethylated in those cases, compared to hypomethylation detected in biallelically expressed samples. Unfortunately, I was unable to determine the parental origin of methylated and unmethylated alleles of *CGB5* due to limited number of informative polymorphisms, and loss of information about their parental origin in bisulfite-treated DNA. Therefore, it is not clear whether *CGB5* promoter methylation results from a methylation allelic polymorphism (MAP) or polymorphic imprinting. MAP is defined as interindividual variation in DNA methylation that is independent of parent-of-origin effect (Pastinen *et al*., 2004; Yuen *et al*., 2009). Polymorphic imprinting involves a subset of individuals that have parent of origin specific silencing of one of the alleles (Naumova and Croteau, 2004). The human genes *WT1* (Wilms' tumor suppressor) and *IGF2R* (insulin-like growth factor 2 receptor) are examples of polymorphic imprinting in placenta (Jinno *et al*., 1994; Xu *et al*., 1993).

Despite the small number of informative *CGB5* cases and limited statistical power, I found an association between recurrent miscarriage and gained *CGB5* promoter MAP in placenta. Notably, several recent studies have found similar associations between gain of methylation of placental genes, pregnancy outcome and infant growth-related complications. Specifically, elevated placental methylation and decreased expression of *WNT2* (wingless-type MMTV integration site family member 2) and *HSD11B2* (hydroxysteroid 11-beta dehydrogenase 2) genes have been found in SGA infants (Ferreira *et al*., 2011; Marsit *et al*., 2012). Increased methylation of *TUSC3* (tumor suppressor candidate 3) promoter associated with preeclampsia (Yuen *et al*., 2009) and higher promoter methylation of glucocorticoid receptor gene (*NR3C1*) was observed in placenta of LGA infants (Filiberto *et al*., 2011). These results illustrate the importance of studying tissue-specific variation of DNA methylation. Such studies will likely increase our understanding of interindividual differences,

complex disease susceptibility, and in the context of this work, potential biomarkers for adverse pregnancy outcome.

There are several possible scenarios to explain the gain of placental methylation that ultimately affects gene expression. First, allele-specific methylation may be sequence-dependent and determined by genetic variants, such SNPs (Shoemaker *et al*., 2010). However this is probably not the case for *CGB5*, since no correlation between DNA methylation profiles and neighbouring SNPs was apparent in my analysis (**Table 1** and **Table 2** in **Ref. III**). Second, aberrant accumulation of methylation may be the result of chromosomal abnormalities (Papageorgiou *et al*., 2011). In fact, Turner syndrome (45, X monosomy) was found in one of the three cases with hemimethylated *CGB5* promoter in this study. Admittedly, the mechanisms of X inactivation and genomic imprinting have evolved in parallel (Reik and Lewis, 2005), suggesting that the entire methylation process may be disturbed in case of Turner syndrome. Third, gain of methylation may also be a result of environmental exposures and conditions in the placental-maternal interface. It may act as a flexible 'emergency exit' tool affecting the implantation process when unfavourable developmental conditions for the offspring are present (Wolf and Hager, 2009). Finally, the detected methylation allelic polymorphism in *CGB5* may also be a tag for methylation-associated functional alteration of one or more undiscovered genes.

The aberrant methylation pattern of *CGB5* promoter may originate from male gametogenesis, in which the genome of primordial germ cells has been completely demethylated, followed by sex-specific *de novo* methylation (Hajkova *et al*., 2002; Kerjean *et al*., 2000; Lee *et al*., 2002). Alternatively, the pattern may originate from pre-implantation embryo stage when the second wave of genome-wide demethylation and remethylation occurs, and is being maintained clonally in each villus tree (Reik *et al*., 2001). In both cases, the skewed methylation pattern has probably arisen due to defects in establishing methylation (Reik *et al*., 2001). The MAP may be established after implantation in first trimester placenta as response to signals from the maternal-fetal interface (Buckberry *et al*., 2012).

5.3. Fine-scale tuning of placental gene expression and link to pregnancy complications

The transcription and translation of placental genes is subject to precise temporal and spatial regulation. Aberrations in these detailed regulation programs could result from irregular implantation and placentation, chromosomal or genomic copy number alterations of total DNA. Such disturbances may lead to various pregnancy complications. Despite the great importance of placenta in mediating the rapid physiological changes in pregnancy, data on temporal dynamics of placental gene expression in normal and complicated pregnancies are limited.

In the first part of this thesis, the expression pattern of *CGB* genes in placenta was determined over three trimesters of normal uncomplicated pregnancy. Despite the high interindividual variation of mRNA transcription of *CGB* genes between samples in the same study groups, we detected a distinct expression pattern of *CGB* genes over the course of pregnancy. Notably, we found the highest total mRNA transcription of *CGB* genes during first trimester, consistent with the rapid increase in HCG hormone production during the same period (Hay, 1988). However, in placental tissue of first trimester pregnancy complications (recurrent miscarriage, ectopic and molar pregnancy) *CGB* genes were characterised by considerably shifted expression profiles. We detected significantly reduced total transcription of *HCGβ*-coding genes in cases of recurrent miscarriage, and increased transcription level in pregnancies diagnosed as molar or ectopic. Interestingly, we observed that the transcription of *CGB* genes and serum hormone levels were not always concordant in pathological pregnancies. Spontaneous abortions are characterised by critically low maternal serum levels of HCG whereas ectopic and molar pregnancies have high serum concentration of HCG. The transcription levels of *CGB* genes confirm these trends (Feng *et al*., 2006; Letterie and Hibbert, 2000). However, the high mRNA expression of *CGB* genes and low presence of HCG in maternal serum is controversial (Sivalingam *et al*., 2011). In addition, elevated expression of maternal HCG hormone but low transcription of *CGB* genes is found in cases of Down syndrome (trisomy 21) (Brizot *et al.*, 1995; Jauniaux et al., 2000). In cases of ectopic pregnancy, in which the embryo was implanted in fallopian tube, low serum HCG level may indicate unstable hormone assembly or misregulated transport. In placentas of recurrent miscarriage, the low expression of *HCGβ* may result from transcriptional or post-transcriptional silencing.

In the second part of my thesis I focused on the transcriptiome of human placenta across three months of early pregnancy. So far only three published studies have compared the expression profile of the full placental transcriptome between tissue material representing different gestational ages (Mikheev *et al*., 2008; Sitras *et al*., 2012; Winn *et al*., 2007). Although understanding the gene expression changes in developing human placenta across the first and the second trimester is very important, only one study before the current study had investigated this issue (Mikheev *et al*., 2008). However, the study design did not include experimental validation and replication of the discovery outcome from microarray analysis. In the current thesis, analysis of data from the GeneChip microarray identified 154 genes with significant change in gene expression over gestational weeks 5 to 18, and found that most of the genes showed increase in transcript levels over this period. I validated 24 top-ranking genes from the microarray analysis using RT-qPCR and extended sample sets of first, second and third trimester placentas. Ten of the analysed genes showed clear expressional peak in mid-pregnancy placentas compared to significantly lower expression in first and third trimester. Notably, several genes with gradual increase in expression from early to mid-gestation are involved in implantation,

regulation of mammalian placental function and embryonic development, also proving the importance of dynamic regulation of these genes in placenta (**Table S9** in **Ref. IV**). For example, *BMP5* (Guenther *et al*., 2008), *CDH11* (Farber *et al*., 2011), *FST* (Gajos-Michniewicz *et al*., 2010) and *STC1* (Yeung *et al*., 2012) are associated with bone and skeleton formation whereas *GATM* (Braissant *et al*., 2005) and *NRCAM* (Grumet, 1991) function in nervous system development. *STC1* is important in kidney development and implantation (Allegra *et al*., 2009; Yeung *et al*., 2012). Contribution of *STC1* to implantation is also found in other mammals like pigs and sheep (Song *et al*., 2006; Song *et al*., 2009). Both *Ccng2* and *Zfp36l1* participate in implantation and placentation in mice (Stumpo *et al*., 2004; Yue *et al*., 2005).

The strength of the current thesis is that in addition to identifying the genes specific to mid-gestation human placenta, I addressed the possible involvement of those genes in the pathogenesis of term complications. I tested our novel hypothesis that late pregnancy complications may be accompanied by abnormal expression of mid-gestation genes at term. For instance, genes that are normally inhibited in term are insufficiently down-regulated in disease, and genes that normally reach the expressional plateau already in mid-gestation are further up-regulated in late pregnancy. The hypothesis was tested comparatively in normal term placentas and placentas from maternal complications (preeclampsia, gestational diabetes mellitus) and fetal complications (overgrowth and growth restriction). Several recent studies have shown associations between altered placental gene expression and pregnancy complications, such as preeclampsia, gestational diabetes, and fetal growth restriction (Enquobahrie *et al.*, 2008; Enquobahrie et al., 2009; Sitras *et al.*, 2009a). Although the symptoms of these conditions manifest in late pregnancy, their pathogenesis is commonly associated with disturbed gene expression and placental development already in early pregnancy (Founds *et al*., 2009).

In this study, the *STC1* gene showed the highest expression in second trimester of normal pregnancy. This gene was also characterised by increased mRNA expression levels in all term pregnancy complication groups compared to controls, and had the most significant effect in PE and SGA groups. These findings are consistent with the reduced fetal growth of transgenic mice over-expressing human *STC1* (Johnston *et al*., 2010). Moreover, in agreement with my mRNA measurements, STC1 protein level was significantly increased in postpartum maternal blood plasma of patients with pregnancy complications (PE, SGA and GDM). Notably, the highest plasma levels were measured for the women with complex pathologies that had simultaneously developed PE and given birth to an SGA baby (median 731 pg/ml vs. 418 pg/ml in controls; P=4.8x10^{-4}). These data support the previous reports about common pathologic origin of preeclampsia and fetal growth restriction due to shallow implantation, failure of trophoblast invasion and missing transformation of the uterine arteries, ultimately resulting in placental hypoxia (Huppertz, 2011; Nishizawa *et al*., 2011). Furthermore, the *STC1* promoter is known to harbour a binding site

for HIF-1 transcription factor that regulates genes involved in cellular response to low oxygen concentrations (Law *et al*., 2010). Preeclampsia causes the greatest damage to the mother's kidneys and is accompanied by impaired renal function (Mirza and Cleary, 2009). Moreover, retarded fetal growth during pregnancy may lead to lifelong reduction in the number of nephrons that in turn may result the hypertensive disorders (Mackenzie and Brenner, 1995). As a link between renal failure and preeclampsia, *STC1* locus has recently been associated to adult chronic kidney disease (Boger *et al*., 2011; Kottgen *et al*., 2009). However, it remains to be found whether the circulating maternal plasma STC1 is partially derived from placenta or it reflects maternal renal protective actions against pathophysiology of preeclampsia (Huang *et al*., 2009). Nevertheless, the observed increase in maternal plasma levels of STC1 in pregnancy complications warrants further investigation of its potential as a prognostic biomarker of pregnancy.

In addition to above, I detected significantly higher gene expression of *LYPD6* (encoding LY6/PLAUR domain containing 6) and *CCNG2* (Cyclin-G2) in pathological placentas (**Table 6**). According to immunohistochemical staining the corresponding proteins showed higher expression in placentas of complicated pregnancies (PE, GDM) compared to normal control placentas (**Figure 8**; **Figure S5** and **Figure S6** in **Ref. IV**). Importantly, this study is the first to demonstrate LYPD6 protein expression in syncytiotrophoblast. In mice, *Lypd6* is known to be highly expressed in the embryonic ovary during mouse gonad development (Chen *et al*., 2012). LYPD6 over-expression is associated with transcriptional regulation through suppression of transcription activator protein AP-1 (Zhang *et al*., 2010). AP-1 is a dimeric protein composed of Jun and Fos proto-oncogenes (Angel and Karin, 1991), and the expression of these genes is known to be decreased in PE and PE-FGR placentas (Marzioni *et al*., 2010). Thus, the involvement of LYPD6 in pathology of pregnancy complications warrants further attention. CCNG2 is a cell cycle inhibitor in response to diverse growth inhibitory signals, such as oxidative stress, DNA damage and differentiation (Bates *et al*., 1996; Bennin *et al*., 2002). Increased CCNG2 expression in pregnancy complications may therefore reflect the local cellular stress conditions.

5.4. Placental gene expression and link to adult disease

The hypothesis of Developmental Origins of Health and Disease (DOHaD) has recently gained increasing attention (Barker *et al*., 2002; Langley-Evans *et al*., 2012). The period from conception to birth and the first years of child's life are considered critical in the context of adult disease susceptibility. The hypothesis is now supported by several epidemiologic studies that have found associations between adult diseases and measurements from early life. For instance, low

birth weight of the infant has been related to increased later life risk of cardiovascular diseases (Barker, 1995), hypertension (Eriksson *et al*., 2000) and type 2 diabetes (Forsen *et al*., 2000). Increased infant birth weight, on the other hand, is associated with various cancer types (Caughey and Michels, 2009; Harder *et al*., 2008; Michels and Xue, 2006; Michos *et al*., 2007). In addition to birth weight, the size and shape of placenta at birth have been suggested as predictors of offspring's chronic diseases in later life (Barker, 2012). Besides features of the newborn, several studies indicate that disorders of maternal metabolism during pregnancy such as gestational diabetes mellitus and preeclampsia, may increase the mother's and child's risk of adulthood diseases and conditions such as heart disease, hypertension, chronic kidney disease, type 2 diabetes, stroke and obesity (Kajantie *et al*., 2009; Sattar and Greer, 2002).

Interestingly, the placental genes detected in the microarray analysis of the current thesis have been also associated with several adult disease conditions. In particular, the group of genes with peak expression in second trimester placentas is interesting from the clinical perspective (**Table 2** and **Table S9** in **Ref. IV**). For example, *CDH11* is associated with the development of osteoarthritis (Karlsson *et al*., 2010), and *GPR183* and *MEG3* are involved in the development of type 1 diabetes (Heinig *et al*., 2010; Wallace *et al*., 2010). *GATM* and *STC1* have been found among the top loci in genome-wide association studies of chorionic kidney disease (Boger *et al*., 2011; Kottgen *et al*., 2009) but are also implicated in heart failure (Cullen *et al*., 2006; Sheikh-Hamad *et al*., 2003). *CCNG2* encodes a cell cycle regulator altered in different cancer types (**Table S9** in **Ref. IV**). Additionally, the *LYPD6* gene may be important in brain neuronal development, as it is located in a microduplication region linked to human developmental delay and autistic features (Chung *et al*., 2012). Clearly, placental genes regulating *in utero* fetal development are also involved in pathogenesis of chronic adult diseases, underlining the importance of placenta in developmental programming (McKay, 2011). However, the mechanisms by witch *in utero* events influence long-term individual health are still poorly understood.

6. CONCLUSIONS

This doctoral thesis focuses on several important and timely research topics in the field of human pregnancy, specifically in placental gene expression. The work is summarised in the following conclusions.

1. The dynamics of placental gene expression during normal human pregnancy was explored with target-gene based approaches as well as exploratory genome-wide techniques. First, focused investigation of the *HCG β-subunit coding* (*CGB*) genes revealed that their placental transcription is highest during the first trimester of human pregnancy, followed by a sharp decline in expression during the second trimester and modest increase in term placenta. This observation is expected, as the HGC genes are crucial for embryo implantation, placentation and maintenance of early pregnancy. Second, I used microarrays to measure gene expression in placenta during first and second trimester of pregnancy (gestational weeks 5 to 18). This study is the first of its kind and it reveals an intricate system of dynamic transcription. An important result of this study is the identification and validation of several mid-pregnancy specific genes with the highest expression at second trimester compared to first and third trimesters. The results of this study will serve as a resource of gene expression data in placenta for further studies focusing on normal or complicated pregnancies.

2. The findings in my doctoral thesis underline the importance of dynamic and fine-scale regulation of placental genes during gestation and in human health. Experiments and data analysis revealed genes whose aberrant expression patterns were informative of pregnancy complications. For instance, recurrent miscarriages in the first trimester are generally associated with critically low levels of HCG hormone. In agreement with the above, I found that the mRNA expression of *HCGβ* genes is significantly lower in placentas of recurrent miscarriages. As another example, several mid-gestation genes identified in the genome-wide screen (*CCNG2*, *STC1*, *GATM*, *GPR183*, *CDH11*, *LYPD6*, *MEG3*) showed aberrant expression patterns in placental samples of pregnancy complications such as preeclampsia, gestational diabetes mellitus and fetal growth restriction. These findings underline the importance of placental gene expression and provide leads for future studies.

3. Epigenetic mechanisms are increasingly acknowledged as major regulators of gene transcription. In this doctoral thesis, I characterised a transcriptional silencing mechanism of pregnancy complication that is driven by DNA methylation. I found that most normal placenta samples showed equal *CGB5* and *CGB8* gene expression from both paternal and maternal alleles as well as hypomethylation of corresponding promoters. In contrast, two recurrent miscarriage samples and one sample of elective termination involved monoallelic (maternal) transcription of *CGB5* gene and promoter hemi-

methylation, suggesting that the pregnancy complication may be driven by aberrant promoter methylation and occasional gain of paternal imprinting (GOI). Further investigation of allele-specific transcription and individual variability of methylation may lead to predictive biomarkers for the intrauterine environment of pregnancy complications.

4. One of the major results of this work is the discovery of the STC1 protein as a potential biomarker of pregnancy complications. STC1 is a secreted glycoprotein hormone that can be easily extracted from mother's blood serum and is therefore a potentially actionable finding. Increased levels of STC1 are in significant association with the restricted fetal growth complication. Further studies on larger sample cohorts are required to establish the protein as a prognostic biomarker.

5. Furthermore, the doctoral work revealed that the poorly studied protein LYPD6 is highly expressed in placental syncytiotrophoblast. Increased expression of cytoplasmic proteins LYPD6 and CCNG2 was detected with immunohistochemical staining in placental samples from patients with preeclampsia and gestational diabetes. The role of these proteins in the placental pathology of pregnancy complications requires further investigation.

6. Aberrant expression of placental genes not only associates with pregnancy complications but also is likely to affect the adult health of the mother as well as the infant. As an additional novel finding, all tested mid-pregnancy specific genes with aberrant expression in pregnancy complications are linked to chronic adult diseases, such as cancer, osteoarthritis, diabetes, heart disease, kidney disease, among others. This observation supports the recent discussion about the role of placenta in developmental programming and *in utero* origin of adult disease.

7. Proper *in utero* development programs the health of mother and her baby during pregnancy but also later in their life. As the growth and aging of the global human population increasingly impact the society, disease prevention and extension of working age are important challenges of future science and medicine. Thus, understanding the role of placenta in healthy and complicated pregnancy has great clinical and societal importance in predicting future adult health.

7. REFERENCES

Aguilera, O., Fernandez, A. F., Munoz, A., Fraga, M. F., 2010. Epigenetics and environment: a complex relationship. J Appl Physiol. 109, 243–51.

Akoum, A., Metz, C. N., Morin, M., 2005. Marked increase in macrophage migration inhibitory factor synthesis and secretion in human endometrial cells in response to human chorionic gonadotropin hormone. J Clin Endocrinol Metab. 90, 2904–10.

Allegra, A., Marino, A., Coffaro, F., Lama, A., Rizza, G., Scaglione, P., Sammartano, F., Santoro, A., Volpes, A., 2009. Is there a uniform basal endometrial gene expression profile during the implantation window in women who became pregnant in a subsequent ICSI cycle? Hum Reprod. 24, 2549–57.

Alsat, E., Guibourdenche, J., Couturier, A., Evain-Brion, D., 1998. Physiological role of human placental growth hormone. Mol Cell Endocrinol. 140, 121–7.

Amaral, P. P., Clark, M. B., Gascoigne, D. K., Dinger, M. E., Mattick, J. S., 2010. lncRNAdb: a reference database for long noncoding RNAs. Nucleic Acids Res. 39, D146–51.

Angel, P., Karin, M., 1991. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta. 1072, 129–57.

Apps, R., Sharkey, A., Gardner, L., Male, V., Trotter, M., Miller, N., North, R., Founds, S., Moffett, A., 2011. Genome-wide expression profile of first trimester villous and extravillous human trophoblast cells. Placenta. 32, 33–43.

Attwood, J. T., Yung, R. L., Richardson, B. C., 2002. DNA methylation and the regulation of gene transcription. Cell Mol. Life Sci. 59, 241–257.

Avila, L., Yuen, R. K., Diego-Alvarez, D., Penaherrera, M. S., Jiang, R., Robinson, W. P., 2010. Evaluating DNA methylation and gene expression variability in the human term placenta. Placenta. 31, 1070–7.

Bailey, W. J., Fitch, D. H., Tagle, D. A., Czelusniak, J., Slightom, J. L., Goodman, M., 1991. Molecular evolution of the psi eta-globin gene locus: gibbon phylogeny and the hominoid slowdown. Mol Biol Evol. 8, 155–84.

Banister, C. E., Koestler, D. C., Maccani, M. A., Padbury, J. F., Houseman, E. A., Marsit, C. J., 2011. Infant growth restriction is associated with distinct patterns of DNA methylation in human placentas. Epigenetics. 6, 920–7.

Barker, D. J., 1995. Fetal origins of coronary heart disease. BMJ. 311, 171–4.

Barker, D. J., 2012. Sir Richard Doll Lecture. Developmental origins of chronic disease. Public Health. 126, 185–9.

Barker, D. J., Eriksson, J. G., Forsen, T., Osmond, C., 2002. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 31, 1235–9.

Bartolomei, M. S., Ferguson-Smith, A. C., 2011. Mammalian genomic imprinting. Cold Spring Harb Perspect Biol. 3.

Bates, S., Rowan, S., Vousden, K. H., 1996. Characterisation of human cyclin G1 and G2: DNA damage inducible genes. Oncogene. 13, 1103–9.

Benirschke, K., Kaufmann, P., Baergen, R., 2006. Placental Types. Pathology of the human placenta. Springer, pp. 30–41.

Benirschke, P., Kaufmann, P., 2000. Pathology of the human placenta. Springer, Berlin.

Bennin, D. A., Don, A. S., Brake, T., McKenzie, J. L., Rosenbaum, H., Ortiz, L., DePaoli-Roach, A. A., Horne, M. C., 2002. Cyclin G2 associates with protein phosphatase 2A catalytic and regulatory B' subunits in active complexes and induces nuclear aberrations and a G1/S phase cell cycle arrest. J Biol Chem. 277, 27449–67.

Bentwich, I., Avniel, A., Karov, Y., Aharonov, R., Gilad, S., Barad, O., Barzilai, A., Einat, P., Einav, U., Meiri, E.*, et al.*, 2005. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet. 37, 766–70.

Berkowitz, R., Ozturk, M., Goldstein, D., Bernstein, M., Hill, L., Wands, J. R., 1989. Human chorionic gonadotropin and free subunits' serum levels in patients with partial and complete hydatidiform moles. Obstet Gynecol. 74, 212–6.

Bird, A., 2002. DNA methylation patterns and epigenetic memory. Genes Dev. 16, 6–21.

Bird, A. P., 1986. CpG-rich islands and the function of DNA methylation. Nature. 321, 209–13.

Blackburn, S. T., 2003. Maternal, fetal and neonatal physiology: a clinical perspective. Saunders, St. Louis, USA.

Blaise, S., de Parseval, N., Benit, L., Heidmann, T., 2003. Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. Proc Natl Acad Sci U S A. 100, 13013–8.

Bo, M., Boime, I., 1992. Identification of the transcriptionally active genes of the chorionic gonadotropin beta gene cluster in vivo. J. Biol. Chem. 267, 3179–3184.

Bogart, M. H., Pandian, M. R., Jones, O. W., 1987. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn. 7, 623–30.

Boger, C. A., Gorski, M., Li, M., Hoffmann, M. M., Huang, C., Yang, Q., Teumer, A., Krane, V., O'Seaghdha, C. M., Kutalik, *Z., et al.*, 2011. Association of eGFR-Related Loci Identified by GWAS with Incident CKD and ESRD. PLoS Genet. 7, e1002292.

Boyd, J. D., 1970. The human placenta. W. Heffer and Sons, Cambridge.

Braissant, O., Henry, H., Villard, A. M., Speer, O., Wallimann, T., Bachmann, C., 2005. Creatine synthesis and transport during rat embryogenesis: spatiotemporal expression of AGAT, GAMT and CT1. BMC Dev Biol. 5, 9.

Bressan, F. F., De Bem, T. H., Perecin, F., Lopes, F. L., Ambrosio, C. E., Meirelles, F. V., Miglino, M. A., 2009. Unearthing the roles of imprinted genes in the placenta. Placenta. 30, 823–834.

Brizot, M. L., Jauniaux, E., McKie, A. T., Farzaneh, F., Nicolaides, K. H., 1995. Placental expression of alpha and beta subunits of human chorionic gonadotrophin in early pregnancies with Down's syndrome. Hum Reprod. 10, 2506–9.

Brown, P., McNeilly, J. R., Wallace, R. M., McNeilly, A. S., Clark, A. J., 1993. Characterization of the ovine LH beta-subunit gene: the promoter directs gonadotrope-specific expression in transgenic mice. Mol Cell Endocrinol. 93, 157–65.

Buckberry, S., Bianco-Miotto, T., Hiendleder, S., Roberts, C. T., 2012. Quantitative Allele-Specific Expression and DNA Methylation Analysis of H19, IGF2 and IGF2R in the Human Placenta across Gestation Reveals H19 Imprinting Plasticity. PLoS One. 7, e51210.

Burton, G. J., Jauniaux, E., Charnock-Jones, D. S., 2010. The influence of the intrauterine environment on human placental development. Int J Dev Biol. 54, 303–12.

Buyalos, R. P., Glassman, L. M., Rifka, S. M., Falk, R. J., Macarthy, P. O., Tyson, V. J., DiMattina, M., 1992. Serum beta-human chorionic gonadotropin, estradiol and progesterone as early predictors of pathologic pregnancy. J Reprod Med. 37, 261–6.

Byun, H. M., Siegmund, K. D., Pan, F., Weisenberger, D. J., Kanel, G., Laird, P. W., Yang, A. S., 2009. Epigenetic profiling of somatic tissues from human autopsy

specimens identifies tissue- and individual-specific DNA methylation patterns. Hum Mol Genet. 18, 4808–17.
Campain, J. A., Gutkin, D. W., Cox, G. S., 1993. Differential DNA methylation of the chorionic gonadotropin beta-subunit multigene family. Mol Endocrinol. 7, 1331–1346.
Carter, A. M., 2007. Animal models of human placentation – a review. Placenta. 28 Suppl A, S41–7.
Castellucci, M., Kosanke, G., Verdenelli, F., Huppertz, B., Kaufmann, P., 2000. Villous sprouting: fundamental mechanisms of human placental development. Hum Reprod Update. 6, 485–94.
Caughey, R. W., Michels, K. B., 2009. Birth weight and childhood leukemia: a meta-analysis and review of the current evidence. Int J Cancer. 124, 2658–70.
Chen, H., Palmer, J. S., Thiagarajan, R. D., Dinger, M. E., Lesieur, E., Chiu, H., Schulz, A., Spiller, C., Grimmond, S. M., Little, M. H.*, et al.*, 2012. Identification of novel markers of mouse fetal ovary development. PLoS One. 7, e41683.
Chen, K., Rajewsky, N., 2007. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet. 8, 93–103.
Cheng, S. L., Lecanda, F., Davidson, M. K., Warlow, P. M., Zhang, S. F., Zhang, L., Suzuki, S., St John, T., Civitelli, R., 1998. Human osteoblasts express a repertoire of cadherins, which are critical for BMP-2-induced osteogenic differentiation. J Bone Miner Res. 13, 633–44.
Chi, H., Qiao, J., Li, H., Liu, P., Ma, C., 2010. Double measurements of serum HCG concentration and its ratio may predict IVF outcome. Reprod Biomed Online. 20, 504–9.
Chim, S. S., Jin, S., Lee, T. Y., Lun, F. M., Lee, W. S., Chan, L. Y., Jin, Y., Yang, N., Tong, Y. K., Leung, T. Y.*, et al.*, 2008. Systematic search for placental DNA-methylation markers on chromosome 21: toward a maternal plasma-based epigenetic test for fetal trisomy 21. Clin Chem. 54, 500–11.
Christensen, B. C., Houseman, E. A., Marsit, C. J., Zheng, S., Wrensch, M. R., Wiemels, J. L., Nelson, H. H., Karagas, M. R., Padbury, J. F., Bueno, R.*, et al.*, 2009. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet. 5, e1000602.
Chung, B. H., Mullegama, S., Marshall, C. R., Lionel, A. C., Weksberg, R., Dupuis, L., Brick, L., Li, C., Scherer, S. W., Aradhya, S.*, et al.*, 2012. Severe intellectual disability and autistic features associated with microduplication 2q23.1. Eur J Hum Genet. 20, 398–403.
Cindrova-Davies, T., Yung, H. W., Johns, J., Spasic-Boskovic, O., Korolchuk, S., Jauniaux, E., Burton, G. J., Charnock-Jones, D. S., 2007. Oxidative stress, gene expression, and protein changes induced in the human placenta during labor. Am J Pathol. 171, 1168–79.
Coan, P. M., Burton, G. J., Ferguson-Smith, A. C., 2005. Imprinted genes in the placenta – a review. Placenta. 26 Suppl A, S10–20.
Cocquebert, M., Berndt, S., Segond, N., Guibourdenche, J., Murthi, P., Aldaz-Carroll, L., Evain-Brion, D., Fournier, T., 2012. Comparative expression of hCG beta-genes in human trophoblast from early and late first-trimester placentas. Am J Physiol Endocrinol Metab. 303, E950–8.
Cole, L. A., 2009. hCG and hyperglycosylated hCG in the establishment and evolution of hemochorial placentation. J Reprod Immunol. 82, 112–8.

Cole, L. A., 2010. Biological functions of hCG and hCG-related molecules. Reprod Biol Endocrinol. 8, 102.
Cole, L. A., 2012a. hCG, the wonder of today's science. Reprod Biol Endocrinol. 10, 24.
Cole, L. A., 2012b. Individual deviations in human chorionic gonadotropin concentrations during pregnancy. Am J Obstet Gynecol. 204, 349 e1–7.
Cole, L. A., 2012c. The hCG assay or pregnancy test. Clin Chem Lab Med. 50, 617–30.
Cole, L. A., Hartle, R. J., Laferla, J. J., Ruddon, R. W., 1983. Detection of the free beta subunit of human chorionic gonadotropin (HCG) in cultures of normal and malignant trophoblast cells, pregnancy sera, and sera of patients with choriocarcinoma. Endocrinology. 113, 1176–8.
Constancia, M., Hemberger, M., Hughes, J., Dean, W., Ferguson-Smith, A., Fundele, R., Stewart, F., Kelsey, G., Fowden, A., Sibley, C.*, et al.*, 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. Nature. 417, 945–8.
Constancia, M., Kelsey, G., Reik, W., 2004. Resourceful imprinting. Nature. 432, 53–57.
Cotton, A. M., Avila, L., Penaherrera, M. S., Affleck, J. G., Robinson, W. P., Brown, C. J., 2009. Inactive X chromosome-specific reduction in placental DNA methylation. Hum Mol Genet. 18, 3544–52.
Cullen, M. E., Yuen, A. H., Felkin, L. E., Smolenski, R. T., Hall, J. L., Grindle, S., Miller, L. W., Birks, E. J., Yacoub, M. H., Barton, P. J., 2006. Myocardial expression of the arginine:glycine amidinotransferase gene is elevated in heart failure and normalized after recovery: potential implications for local creatine synthesis. Circulation. 114, I16–20.
Davies, W., Isles, A. R., Wilkinson, L. S., 2005. Imprinted gene expression in the brain. Neurosci Biobehav Rev. 29, 421–30.
Diplas, A. I., Lambertini, L., Lee, M. J., Sperling, R., Lee, Y. L., Wetmur, J., Chen, J., 2009. Differential expression of imprinted genes in normal and IUGR human placentas. Epigenetics. 4, 235–40.
Donker, R. B., Mouillet, J. F., Chu, T., Hubel, C. A., Stolz, D. B., Morelli, A. E., Sadovsky, Y., 2012. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol Hum Reprod. 18, 417–24.
Du, X., Rousseau, M., Ounissi-Benkalha, H., Marchand, L., Jetha, A., Paraskevas, S., Goodyer, C., Polychronakos, C., 2011. Differential expression pattern of ZAC in developing mouse and human pancreas. J Mol Histol. 42, 129-36.
Dupressoir, A., Marceau, G., Vernochet, C., Benit, L., Kanellopoulos, C., Sapin, V., Heidmann, T., 2005. Syncytin-A and syncytin-B, two fusogenic placenta-specific murine envelope genes of retroviral origin conserved in Muridae. Proc Natl Acad Sci U S A. 102, 725–30.
Edelstam, G., Karlsson, C., Westgren, M., Lowbeer, C., Swahn, M. L., 2007. Human chorionic gonadatropin (hCG) during third trimester pregnancy. Scand J Clin Lab Invest. 67, 519–25.
Enders, A. C., 2009. Reasons for diversity of placental structure. Placenta. 30 Suppl A, S15–8.
Enders, A. C., Carter, A. M., 2004. What can comparative studies of placental structure tell us? – A review. Placenta. 25 Suppl A, S3–9.
Enquobahrie, D. A., Meller, M., Rice, K., Psaty, B. M., Siscovick, D. S., Williams, M. A., 2008. Differential placental gene expression in preeclampsia. Am J Obstet Gynecol. 199, 566 e1–11.

Enquobahrie, D. A., Williams, M. A., Qiu, C., Meller, M., Sorensen, T. K., 2009. Global placental gene expression in gestational diabetes mellitus. Am J Obstet Gynecol. 200, 206 e1–13.

Eriksson, J., Forsen, T., Tuomilehto, J., Osmond, C., Barker, D., 2000. Fetal and childhood growth and hypertension in adult life. Hypertension. 36, 790–4.

Eta, E., Ambrus, G., Rao, C. V., 1994. Direct regulation of human myometrial contractions by human chorionic gonadotropin. J Clin Endocrinol Metab. 79, 1582–6.

Ewen, K. A., Koopman, P., 2010. Mouse germ cell development: from specification to sex determination. Mol Cell Endocrinol. 323, 76–93.

Ezashi, T., Hirai, T., Kato, T., Wakabayashi, K., Kato, Y., 1990. The gene for the beta subunit of porcine LH: clusters of GC boxes and CACCC elements. J Mol Endocrinol. 5, 137–46.

Farber, C. R., Kelly, S. A., Baruch, E., Yu, D., Hua, K., Nehrenberg, D. L., de Villena, F. P., Buus, R. J., Garland, T., Jr., Pomp, D., 2011. Identification of quantitative trait loci influencing skeletal architecture in mice: emergence of Cdh11 as a primary candidate gene regulating femoral morphology. J Bone Miner Res. 26, 2174–83.

Feng, H. C., Tsao, S. W., Ngan, H. Y., Kwan, H. S., Shih, S. M., Xue, W. C., Chiu, P. M., Chan, K. W., Cheung, A. N., 2006. Differential gene expression identified in complete hydatidiform mole by combining suppression subtractive hybridization and cDNA microarray. Placenta. 27, 521–526.

Ferguson-Smith, A. C., Surani, M. A., 2001. Imprinting and the epigenetic asymmetry between parental genomes. Science. 293, 1086–9.

Ferreira, J. C., Choufani, S., Grafodatskaya, D., Butcher, D. T., Zhao, C., Chitayat, D., Shuman, C., Kingdom, J., Keating, S., Weksberg, R., 2011. WNT2 promoter methylation in human placenta is associated with low birthweight percentile in the neonate. Epigenetics. 6, 440–9.

Filiberto, A. C., Maccani, M. A., Koestler, D., Wilhelm-Benartzi, C., Avissar-Whiting, M., Banister, C. E., Gagne, L. A., Marsit, C. J., 2011. Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics. 6, 566–72.

Forsen, T., Eriksson, J., Tuomilehto, J., Reunanen, A., Osmond, C., Barker, D., 2000. The fetal and childhood growth of persons who develop type 2 diabetes. Ann Intern Med. 133, 176–82.

Founds, S. A., Conley, Y. P., Lyons-Weiler, J. F., Jeyabalan, A., Hogge, W. A., Conrad, K. P., 2009. Altered global gene expression in first trimester placentas of women destined to develop preeclampsia. Placenta. 30, 15–24.

Frank, D., Fortino, W., Clark, L., Musalo, R., Wang, W., Saxena, A., Li, C. M., Reik, W., Ludwig, T., Tycko, B., 2002. Placental overgrowth in mice lacking the imprinted gene Ipl. Proc Natl Acad Sci U S A. 99, 7490–5.

Freemark, M., 2006. Regulation of maternal metabolism by pituitary and placental hormones: roles in fetal development and metabolic programming. Horm Res. 65 Suppl 3, 41–9.

Fuke, C., Shimabukuro, M., Petronis, A., Sugimoto, J., Oda, T., Miura, K., Miyazaki, T., Ogura, C., Okazaki, Y., Jinno, Y., 2004. Age related changes in 5–methylcytosine content in human peripheral leukocytes and placentas: an HPLC-based study. Ann Hum Genet. 68, 196–204.

Gajos-Michniewicz, A., Piastowska, A. W., Russell, J. A., Ochedalski, T., 2010. Follistatin as a potent regulator of bone metabolism. Biomarkers. 15, 563–74.

Gama-Sosa, M. A., Wang, R. Y., Kuo, K. C., Gehrke, C. W., Ehrlich, M., 1983. The 5-methylcytosine content of highly repeated sequences in human DNA. Nucleic Acids Res. 11, 3087–95.

Gao, Y., Zhang, H. D., Lin, J. S., Zhang, M. P., Zhang, R. G., 2010. [The imprinting status of genetic imprinted gene PEG10 in human hepatocellular carcinomas]. Zhonghua Gan Zang Bing Za Zhi. 18, 894–9.

George, D. L., Phillips, J. A., 3rd, Francke, U., Seeburg, P. H., 1981. The genes for growth hormone and chorionic somatomammotropin are on the long arm of human chromosome 17 in region q21 to qter. Hum Genet. 57, 138–41.

Gicquel, C., Rossignol, S., Cabrol, S., Houang, M., Steunou, V., Barbu, V., Danton, F., Thibaud, N., Le Merrer, M., Burglen, L*., et al.*, 2005. Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. Nat Genet. 37, 1003–7.

Goldsmith, P. C., McGregor, W. G., Raymoure, W. J., Kuhn, R. W., Jaffe, R. B., 1983. Cellular localization of chorionic gonadotropin in human fetal kidney and liver. J Clin Endocrinol Metab. 57, 654–61.

Grigoriu, A., Ferreira, J. C., Choufani, S., Baczyk, D., Kingdom, J., Weksberg, R., 2011. Cell specific patterns of methylation in the human placenta. Epigenetics. 6, 368–79.

Grumet, M., 1991. Cell adhesion molecules and their subgroups in the nervous system. Curr Opin Neurobiol. 1, 370–6.

Gude, N. M., Roberts, C. T., Kalionis, B., King, R. G., 2004. Growth and function of the normal human placenta. Thromb Res. 114, 397–407.

Guenther, C., Pantalena-Filho, L., Kingsley, D. M., 2008. Shaping skeletal growth by modular regulatory elements in the Bmp5 gene. PLoS Genet. 4, e1000308.

Gurbuz, A., Karateke, A., Mengulluoglu, M., Gedikbasi, A., Ozturkmen, M., Kabaca, C., Sahinoglu, Z., 2004. Can serum HCG values be used in the differential diagnosis of pregnancy complicated by hypertension? Hypertens Pregnancy. 23, 1–12.

Haig, D., 1993. Genetic conflicts in human pregnancy. Q Rev Biol. 68, 495–532.

Haig, D., 1996. Altercation of generations: genetic conflicts of pregnancy. Am J Reprod Immunol. 35, 226–32.

Haig, D., 2008. Placental growth hormone-related proteins and prolactin-related proteins. Placenta. 29 Suppl A, S36–41.

Hajkova, P., Erhardt, S., Lane, N., Haaf, T., El-Maarri, O., Reik, W., Walter, J., Surani, M. A., 2002. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 117, 15–23.

Hallast, P., Laan, M., Evolution of the Chorionic Gonadotropin β Genes in Primates. eLS. John Wiley & Sons Ltd, Chichester, 2009.

Hallast, P., Nagirnaja, L., Margus, T., Laan, M., 2005. Segmental duplications and gene conversion: Human luteinizing hormone/chorionic gonadotropin beta gene cluster. Genome Res. 15, 1535–1546.

Hallast, P., Rull, K., Laan, M., 2007. The evolution and genomic landscape of CGB1 and CGB2 genes. Mol Cell Endocrinol. 260–262, 2–11.

Hallast, P., Saarela, J., Palotie, A., Laan, M., 2008. High divergence in primate-specific duplicated regions: human and chimpanzee chorionic gonadotropin beta genes. BMC Evol. Biol. 8, 195.

Hammond, S. M., Caudy, A. A., Hannon, G. J., 2001. Post-transcriptional gene silencing by double-stranded RNA. Nat Rev Genet. 2, 110–9.

Handwerger, S., Freemark, M., 2000. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J Pediatr Endocrinol Metab. 13, 343–56.

Harder, T., Plagemann, A., Harder, A., 2008. Birth weight and subsequent risk of childhood primary brain tumors: a meta-analysis. Am J Epidemiol. 168, 366–73.

Hay, D. L., 1988. Placental histology and the production of human choriogonadotrophin and its subunits in pregnancy. Br. J. Obstet. Gynaecol. 95, 1268–1275.

Hay, W. W., Jr., 1994. Placental transport of nutrients to the fetus. Horm Res. 42, 215–22.

Heinig, M., Petretto, E., Wallace, C., Bottolo, L., Rotival, M., Lu, H., Li, Y., Sarwar, R., Langley, S. R., Bauerfeind, A*., et al.*, 2010. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature. 467, 460–4.

Hiby, S. E., Lough, M., Keverne, E. B., Surani, M. A., Loke, Y. W., King, A., 2001. Paternal monoallelic expression of PEG3 in the human placenta. Hum Mol Genet. 10, 1093–1100.

Hishida, T., Naito, K., Osada, S., Nishizuka, M., Imagawa, M., 2007. peg10, an imprinted gene, plays a crucial role in adipocyte differentiation. FEBS Lett. 581, 4272–8.

Huang, L., Garcia, G., Lou, Y., Zhou, Q., Truong, L. D., DiMattia, G., Lan, X. R., Lan, H. Y., Wang, Y., Sheikh-Hamad, D., 2009. Anti-inflammatory and renal protective actions of stanniocalcin-1 in a model of anti-glomerular basement membrane glomerulonephritis. Am J Pathol. 174, 1368–78.

Huppertz, B., 2011. Placental pathology in pregnancy complications. Thromb Res. 127 Suppl 3, S96–9.

Jaenisch, R., Bird, A., 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 33 Suppl, 245–54.

Jameson, J. L., Lindell, C. M., Habener, J. F., 1986. Evolution of different transcriptional start sites in the human luteinizing hormone and chorionic gonadotropin beta-subunit genes. DNA. 5, 227–34.

Jameson, L., Chin, W. W., Hollenberg, A. N., Chang, A. S., Habener, J. F., 1984. The gene encoding the beta-subunit of rat luteinizing hormone. Analysis of gene structure and evolution of nucleotide sequence. J Biol Chem. 259, 15474–80.

Jauniaux, E., Bao, S., Eblen, A., Li, X., Lei, Z. M., Meuris, S., Rao, C. V., 2000. HCG concentration and receptor gene expression in placental tissue from trisomy 18 and 21. Mol Hum Reprod. 6, 5–10.

Jauniaux, E., Poston, L., Burton, G. J., 2006. Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Hum Reprod Update. 12, 747–55.

Jellinek, D. A., Chang, A. C., Larsen, M. R., Wang, X., Robinson, P. J., Reddel, R. R., 2000. Stanniocalcin 1 and 2 are secreted as phosphoproteins from human fibrosarcoma cells. Biochem J. 350 Pt 2, 453–61.

Jinno, Y., Yun, K., Nishiwaki, K., Kubota, T., Ogawa, O., Reeve, A. E., Niikawa, N., 1994. Mosaic and polymorphic imprinting of the WT1 gene in humans. Nat Genet. 6, 305–309.

Jirtle, R. J., 2012. GeneImprint website, http://www.geneimprint.org/.

Johnson, W., Jameson, J. L., 1999. AP-2 (activating protein 2) and Sp1 (selective promoter factor 1) regulatory elements play distinct roles in the control of basal activity and cyclic adenosine 3',5'-monophosphate responsiveness of the human chorionic gonadotropin-beta promoter. Mol Endocrinol. 13, 1963–1975.

Johnston, J., Ramos-Valdes, Y., Stanton, L. A., Ladhani, S., Beier, F., Dimattia, G. E., 2010. Human stanniocalcin-1 or -2 expressed in mice reduces bone size and severely inhibits cranial intramembranous bone growth. Transgenic Res. 19, 1017–39.

Jones, P. A., Takai, D., 2001. The role of DNA methylation in mammalian epigenetics. Science. 293, 1068–70.

Kajantie, E., Eriksson, J. G., Osmond, C., Thornburg, K., Barker, D. J., 2009. Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study. Stroke. 40, 1176–80.

Karlsson, C., Dehne, T., Lindahl, A., Brittberg, M., Pruss, A., Sittinger, M., Ringe, J., 2010. Genome-wide expression profiling reveals new candidate genes associated with osteoarthritis. Osteoarthritis Cartilage. 18, 581–92.

Kaufmann, P., Baergen, R., 2006. Early Development of the Human Placenta. Pathology of Human Placenta. pp. 44–49.

Kerjean, A., Dupont, J. M., Vasseur, C., Le Tessier, D., Cuisset, L., Paldi, A., Jouannet, P., Jeanpierre, M., 2000. Establishment of the paternal methylation imprint of the human H19 and MEST/PEG1 genes during spermatogenesis. Hum Mol Genet. 9, 2183–7.

Khan, M. A., Mittal, S., Kumar, S., Sengupta, J., Ghosh, D., 2010. Quantitative analysis of six gene products as candidate markers of early placental villi development in the human. Indian J Physiol Pharmacol. 54, 299–308.

Kim, S. J., Lee, S. Y., Lee, C., Kim, I., An, H. J., Kim, J. Y., Baek, K. H., Kim, E. J., Kim, J. M., Lee, J. B.*, et al.*, 2006. Differential expression profiling of genes in a complete hydatidiform mole using cDNA microarray analysis. Gynecol Oncol. 103, 654–60.

King, B. F., 1993. Development and structure of the placenta and fetal membranes of nonhuman primates. J Exp Zool. 266, 528–40.

Kliman, H. J., 2000. Uteroplacental blood flow. The story of decidualization, menstruation, and trophoblast invasion. Am J Pathol. 157, 1759–68.

Kokawa, K., Shikone, T., Nakano, R., 1998. Apoptosis in human chorionic villi and decidua in normal and ectopic pregnancy. Mol Hum Reprod. 4, 87–91.

Kottgen, A., Glazer, N. L., Dehghan, A., Hwang, S. J., Katz, R., Li, M., Yang, Q., Gudnason, V., Launer, L. J., Harris, T. B.*, et al.*, 2009. Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet. 41, 712–7.

Kumar, T. R., Matzuk, M. M., 1995. Cloning of the mouse gonadotropin beta-subunit-encoding genes, II. Structure of the luteinizing hormone beta-subunit-encoding genes. Gene. 166, 335–336.

Lambertini, L., Diplas, A. I., Lee, M. J., Sperling, R., Chen, J., Wetmur, J., 2008. A sensitive functional assay reveals frequent loss of genomic imprinting in human placenta. Epigenetics. 3, 261–269.

Langley-Evans, S. C., Alexander, B., McArdle, H. J., Sloboda, D. M., 2012. Developmental origins of health and disease. J Nutr Metab. 2012, 838640.

Lanoix, D., Lacasse, A. A., St-Pierre, J., Taylor, S. C., Ethier-Chiasson, M., Lafond, J., Vaillancourt, C., 2012. Quantitative PCR pitfalls: the case of the human placenta. Mol Biotechnol. 52, 234–43.

Lapaire, O., Grill, S., Lalevee, S., Kolla, V., Hosli, I., Hahn, S., 2012. Microarray screening for novel preeclampsia biomarker candidates. Fetal Diagn Ther. 31, 147–53.

Law, A. Y., Ching, L. Y., Lai, K. P., Wong, C. K., 2010. Identification and characterization of the hypoxia-responsive element in human stanniocalcin-1 gene. Mol Cell Endocrinol. 314, 118–27.

Lee, J., Inoue, K., Ono, R., Ogonuki, N., Kohda, T., Kaneko-Ishino, T., Ogura, A., Ishino, F., 2002. Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. Development. 129, 1807–17.

Lee, K. J., Shim, S. H., Kang, K. M., Kang, J. H., Park, D. Y., Kim, S. H., Farina, A., Shim, S. S., Cha, D. H., 2010. Global gene expression changes induced in the human placenta during labor. Placenta. 31, 698–704.

Lefebvre, L., Viville, S., Barton, S. C., Ishino, F., Keverne, E. B., Surani, M. A., 1998. Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. Nat Genet. 20, 163–9.

Leighton, P. A., Ingram, R. S., Eggenschwiler, J., Efstratiadis, A., Tilghman, S. M., 1995. Disruption of imprinting caused by deletion of the H19 gene region in mice. Nature. 375, 34–9.

Letterie, G. S., Hibbert, M., 2000. Serial serum human chorionic gonadotropin (hCG) levels in ectopic pregnancy and first trimester miscarriage. Arch Gynecol Obstet. 263, 168–9.

Li, E., 2002. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet. 3, 662–73.

Li, L., Keverne, E. B., Aparicio, S. A., Ishino, F., Barton, S. C., Surani, M. A., 1999. Regulation of maternal behavior and offspring growth by paternally expressed Peg3. Science. 284, 330–3.

Li, Y., Ye, C., Shi, P., Zou, X. J., Xiao, R., Gong, Y. Y., Zhang, Y. P., 2005. Independent origin of the growth hormone gene family in New World monkeys and Old World monkeys/hominoids. J Mol Endocrinol. 35, 399–409.

Licht, P., Russu, V., Wildt, L., 2001. On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. Semin Reprod Med. 19, 37–47.

Mackenzie, H. S., Brenner, B. M., 1995. Fewer nephrons at birth: a missing link in the etiology of essential hypertension? Am J Kidney Dis. 26, 91–8.

MacLeod, J. N., Lee, A. K., Liebhaber, S. A., Cooke, N. E., 1992. Developmental control and alternative splicing of the placentally expressed transcripts from the human growth hormone gene cluster. J Biol Chem. 267, 14219–26.

Malassine, A., Frendo, J. L., Evain-Brion, D., 2003. A comparison of placental development and endocrine functions between the human and mouse model. Hum Reprod Update. 9, 531–9.

Maltepe, E., Bakardjiev, A. I., Fisher, S. J., 2010. The placenta: transcriptional, epigenetic, and physiological integration during development. J Clin Invest. 120, 1016–25.

Mannik, J., Vaas, P., Rull, K., Teesalu, P., Laan, M., 2012. Differential placental expression profile of human Growth Hormone/Chorionic Somatomammotropin genes in pregnancies with pre-eclampsia and gestational diabetes mellitus. Mol Cell Endocrinol. 355, 180–7.

Männik, J., Vaas, P., Rull, K., Teesalu, P., Rebane, T., Laan, M., 2010. Differential expression profile of growth hormone/chorionic somatomammotropin genes in placenta of small- and large-for-gestational-age newborns. J Clin Endocrinol Metab. 95, 2433–42.

Marsit, C. J., Maccani, M. A., Padbury, J. F., Lester, B. M., 2012. Placental 11-beta hydroxysteroid dehydrogenase methylation is associated with newborn growth and a measure of neurobehavioral outcome. PLoS One. 7, e33794.

Marzioni, D., Todros, T., Cardaropoli, S., Rolfo, A., Lorenzi, T., Ciarmela, P., Romagnoli, R., Paulesu, L., Castellucci, M., 2010. Activating protein-1 family of transcription factors in the human placenta complicated by preeclampsia with and without fetal growth restriction. Placenta. 31, 919–27.

Maston, G. A., Ruvolo, M., 2002. Chorionic gonadotropin has a recent origin within primates and an evolutionary history of selection. Mol. Biol. Evol. 19, 320–335.

Mayer, W., Hemberger, M., Frank, H. G., Grummer, R., Winterhager, E., Kaufmann, P., Fundele, R., 2000a. Expression of the imprinted genes MEST/Mest in human and murine placenta suggests a role in angiogenesis. Dev Dyn. 217, 1–10.

Mayer, W., Niveleau, A., Walter, J., Fundele, R., Haaf, T., 2000b. Demethylation of the zygotic paternal genome. Nature. 403, 501–2.

McKay, R., 2011. Developmental biology: Remarkable role for the placenta. Nature. 472, 298–9.

McLean, M., Bisits, A., Davies, J., Woods, R., Lowry, P., Smith, R., 1995. A placental clock controlling the length of human pregnancy. Nat Med. 1, 460–3.

McLellan, A. S., Fischer, B., Dveksler, G., Hori, T., Wynne, F., Ball, M., Okumura, K., Moore, T., Zimmermann, W., 2005. Structure and evolution of the mouse pregnancy-specific glycoprotein (Psg) gene locus. BMC Genomics. 6, 4.

McMinn, J., Wei, M., Schupf, N., Cusmai, J., Johnson, E. B., Smith, A. C., Weksberg, R., Thaker, H. M., Tycko, B., 2006. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta. 27, 540–9.

Meller, M., Vadachkoria, S., Luthy, D. A., Williams, M. A., 2005. Evaluation of housekeeping genes in placental comparative expression studies. Placenta. 26, 601–7.

Mercer, R. E., Kwolek, E. M., Bischof, J. M., van Eede, M., Henkelman, R. M., Wevrick, R., 2009. Regionally reduced brain volume, altered serotonin neurochemistry, and abnormal behavior in mice null for the circadian rhythm output gene Magel2. Am J Med Genet B Neuropsychiatr Genet. 150B, 1085–99.

Mercer, R. E., Wevrick, R., 2009. Loss of magel2, a candidate gene for features of Prader-Willi syndrome, impairs reproductive function in mice. PLoS One. 4, e4291.

Michels, K. B., Xue, F., 2006. Role of birthweight in the etiology of breast cancer. Int J Cancer. 119, 2007–25.

Michos, A., Xue, F., Michels, K. B., 2007. Birth weight and the risk of testicular cancer: a meta-analysis. Int J Cancer. 121, 1123–31.

Mikheev, A. M., Nabekura, T., Kaddoumi, A., Bammler, T. K., Govindarajan, R., Hebert, M. F., Unadkat, J. D., 2008. Profiling gene expression in human placentae of different gestational ages: an OPRU Network and UW SCOR Study. Reprod Sci. 15, 866–77.

Miller-Lindholm, A. K., LaBenz, C. J., Ramey, J., Bedows, E., Ruddon, R. W., 1997. Human chorionic gonadotropin-beta gene expression in first trimester placenta. Endocrinology. 138, 5459–5465.

Mirza, F. G., Cleary, K. L., 2009. Pre-eclampsia and the kidney. Semin Perinatol. 33, 173–8.

Moffett, A., Loke, C., 2006. Immunology of placentation in eutherian mammals. Nat Rev Immunol. 6, 584–94.

Mohammad, F., Pandey, G. K., Mondal, T., Enroth, S., Redrup, L., Gyllensten, U., Kanduri, C., 2012. Long noncoding RNA-mediated maintenance of DNA methylation and transcriptional gene silencing. Development. 139, 2792–803.

Moore, T., Haig, D., 1991. Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet. 7, 45–49.

Morgan, H. D., Santos, F., Green, K., Dean, W., Reik, W., 2005. Epigenetic reprogramming in mammals. Hum Mol Genet. 14 Spec No 1, R47–58.

Morison, I. M., Paton, C. J., Cleverley, S. D., 2001. The imprinted gene and parent-of-origin effect database. Nucleic Acids Res. 29, 275–6.

Morison, I. M., Ramsay, J. P., Spencer, H. G., 2005. A census of mammalian imprinting. Trends Genet. 21, 457–465.

Murthi, P., Fitzpatrick, E., Borg, A. J., Donath, S., Brennecke, S. P., Kalionis, B., 2008. GAPDH, 18S rRNA and YWHAZ are suitable endogenous reference genes for relative gene expression studies in placental tissues from human idiopathic fetal growth restriction. Placenta. 29, 798–801.

Nakashima, A., Shima, T., Inada, K., Ito, M., Saito, S., 2012. The balance of the immune system between T cells and NK cells in miscarriage. Am J Reprod Immunol. 67, 304–10.

Naumova, A. K., Croteau, S., 2004. Mechanisms of Epigenetic Variation: Polymorphic Imprinting. Curr Genomics. 5, 417–429.

Nelissen, E. C., van Montfoort, A. P., Dumoulin, J. C., Evers, J. L., 2011. Epigenetics and the placenta. Hum Reprod Update. 17, 397–417.

Nishizawa, H., Ota, S., Suzuki, M., Kato, T., Sekiya, T., Kurahashi, H., Udagawa, Y., 2011. Comparative gene expression profiling of placentas from patients with severe pre-eclampsia and unexplained fetal growth restriction. Reprod Biol Endocrinol. 9, 107.

Noguer-Dance, M., Abu-Amero, S., Al-Khtib, M., Lefevre, A., Coullin, P., Moore, G. E., Cavaille, J., 2010. The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. Hum Mol Genet. 19, 3566–82.

Non, A. L., Binder, A. M., Barault, L., Rancourt, R. C., Kubzansky, L. D., Michels, K. B., 2012. DNA methylation of stress-related genes and LINE-1 repetitive elements across the healthy human placenta. Placenta. 33, 183–7.

Novakovic, B., Saffery, R., 2012. The ever growing complexity of placental epigenetics – role in adverse pregnancy outcomes and fetal programming. Placenta. 33, 959–70.

Novakovic, B., Yuen, R. K., Gordon, L., Penaherrera, M. S., Sharkey, A., Moffett, A., Craig, J. M., Robinson, W. P., Saffery, R., 2011. Evidence for widespread changes in promoter methylation profile in human placenta in response to increasing gestational age and environmental/stochastic factors. BMC Genomics. 12, 529.

O'Shaughnessy, P. J., Baker, P. J., Johnston, H., 2006. The foetal Leydig cell –differentiation, function and regulation. Int J Androl. 29, 90–5; discussion 105–8.

Older Aguilar, A. M., Guethlein, L. A., Adams, E. J., Abi-Rached, L., Moesta, A. K., Parham, P., 2010. Coevolution of killer cell Ig-like receptors with HLA-C to become the major variable regulators of human NK cells. J Immunol. 185, 4238–51.

Ono, R., Nakamura, K., Inoue, K., Naruse, M., Usami, T., Wakisaka-Saito, N., Hino, T., Suzuki-Migishima, R., Ogonuki, N., Miki, H.*, et al.*, 2006. Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. Nat Genet. 38, 101–6.

Otsuka, S., Maegawa, S., Takamura, A., Kamitani, H., Watanabe, T., Oshimura, M., Nanba, E., 2009. Aberrant promoter methylation and expression of the imprinted PEG3 gene in glioma. Proc Jpn Acad Ser B Phys Biol Sci. 85, 157–65.

Papageorgiou, E. A., Fiegler, H., Rakyan, V., Beck, S., Hulten, M., Lamnissou, K., Carter, N. P., Patsalis, P. C., 2009. Sites of differential DNA methylation between placenta and peripheral blood: molecular markers for noninvasive prenatal diagnosis of aneuploidies. Am J Pathol. 174, 1609–18.

Papageorgiou, E. A., Karagrigoriou, A., Tsaliki, E., Velissariou, V., Carter, N. P., Patsalis, P. C., 2011. Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. Nat Med. 17, 510–3.

Papper, Z., Jameson, N. M., Romero, R., Weckle, A. L., Mittal, P., Benirschke, K., Santolaya-Forgas, J., Uddin, M., Haig, D., Goodman, M*., et al.*, 2009. Ancient origin of placental expression in the growth hormone genes of anthropoid primates. Proc Natl Acad Sci U S A. 106, 17083–8.

Parrott, A. M., Sriram, G., Liu, Y., Mathews, M. B., 2011. Expression of type II chorionic gonadotropin genes supports a role in the male reproductive system. Mol Cell Biol. 31, 287–99.

Pastinen, T., Sladek, R., Gurd, S., Sammak, A., Ge, B., Lepage, P., Lavergne, K., Villeneuve, A., Gaudin, T., Brandstrom, H*., et al.*, 2004. A survey of genetic and epigenetic variation affecting human gene expression. Physiol Genomics. 16, 184–93.

Patel, P., Boyd, C. A., Johnston, D. G., Williamson, C., 2002. Analysis of GAPDH as a standard for gene expression quantification in human placenta. Placenta. 23, 697–8.

Penaherrera, M. S., Jiang, R., Avila, L., Yuen, R. K., Brown, C. J., Robinson, W. P., 2012. Patterns of placental development evaluated by X chromosome inactivation profiling provide a basis to evaluate the origin of epigenetic variation. Hum Reprod. 27, 1745–53.

Petronella, N., Drouin, G., 2011. Gene conversions in the growth hormone gene family of primates: stronger homogenizing effects in the Hominidae lineage. Genomics. 98, 173–81.

Pierce, J. G., Parsons, T. F., 1981. Glycoprotein hormones: structure and function. Annu Rev Biochem. 50, 465–495.

Poikkeus, P., Hiilesmaa, V., Tiitinen, A., 2002. Serum HCG 12 days after embryo transfer in predicting pregnancy outcome. Hum. Reprod. 17, 1901–1905.

Policastro, P. F., Daniels-McQueen, S., Carle, G., Boime, I., 1986. A map of the hCG beta-LH beta gene cluster. J Biol Chem. 261, 5907–16.

Pozharny, Y., Lambertini, L., Ma, Y., Ferrara, L., Litton, C. G., Diplas, A., Jacobs, A. R., Chen, J., Stone, J. L., Wetmur, J*., et al.*, 2010. Genomic loss of imprinting in first-trimester human placenta. Am J Obstet Gynecol. 202, 391 e1–8.

Rakyan, V. K., Down, T. A., Thorne, N. P., Flicek, P., Kulesha, E., Graf, S., Tomazou, E. M., Backdahl, L., Johnson, N., Herberth, M*., et al.*, 2008. An integrated resource for genome-wide identification and analysis of human tissue-specific differentially methylated regions (tDMRs). Genome Res. 18, 1518–29.

Rao, C. V., Lei, Z. M., 2007. The past, present and future of nongonadal LH/hCG actions in reproductive biology and medicine. Mol Cell Endocrinol. 269, 2–8.

Rawn, S. M., Cross, J. C., 2008. The evolution, regulation, and function of placenta-specific genes. Annu Rev Cell Dev Biol. 24, 159–81.

Reik, W., Dean, W., Walter, J., 2001. Epigenetic reprogramming in mammalian development. Science. 293, 1089–93.

Reik, W., Lewis, A., 2005. Co-evolution of X-chromosome inactivation and imprinting in mammals. Nat Rev Genet. 6, 403–410.
Reik, W., Walter, J., 2001. Genomic imprinting: parental influence on the genome. Nat Rev Genet. 2, 21–32.
Reimand, J., Arak, T., Vilo, J., 2011. g:Profiler – a web server for functional interpretation of gene lists (2011 update). Nucleic Acids Res. 39, W307–15.
Reiss, D., Zhang, Y., Mager, D. L., 2007. Widely variable endogenous retroviral methylation levels in human placenta. Nucleic Acids Res. 35, 4743–54.
Relaix, F., Wei, X., Li, W., Pan, J., Lin, Y., Bowtell, D. D., Sassoon, D. A., Wu, X., 2000. Pw1/Peg3 is a potential cell death mediator and cooperates with Siah1a in p53-mediated apoptosis. Proc Natl Acad Sci U S A. 97, 2105–10.
Rice, J. C., Allis, C. D., 2001. Histone methylation versus histone acetylation: new insights into epigenetic regulation. Curr Opin Cell Biol. 13, 263–73.
Robinson, B. G., Arbiser, J. L., Emanuel, R. L., Majzoub, J. A., 1989. Species-specific placental corticotropin releasing hormone messenger RNA and peptide expression. Mol Cell Endocrinol. 62, 337–41.
Rodesch, F., Simon, P., Donner, C., Jauniaux, E., 1992. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol. 80, 283–5.
Rougier, N., Bourc'his, D., Gomes, D. M., Niveleau, A., Plachot, M., Paldi, A., Viegas-Pequignot, E., 1998. Chromosome methylation patterns during mammalian preimplantation development. Genes Dev. 12, 2108–13.
Rudert, F., Zimmermann, W., Thompson, J. A., 1989. Intra- and interspecies analyses of the carcinoembryonic antigen (CEA) gene family reveal independent evolution in primates and rodents. J Mol Evol. 29, 126–34.
Rull, K., Laan, M., 2005. Expression of beta-subunit of HCG genes during normal and failed pregnancy. Hum Reprod. 20, 3360–3368.
Rull, K., Nagirnaja, L., Laan, M., 2012. Genetics of recurrent miscarriage: challenges, current knowledge, future directions. Front Genet. 3, 34.
Rull, K., Tomberg, K., Koks, S., Mannik, J., Mols, M., Sirotkina, M., Varv, S., Laan, M., 2013. Increased placental expression and maternal serum levels of apoptosis-inducing TRAIL in recurrent miscarriage. Placenta. 34, 141–8.
Sasaki, H., Matsui, Y., 2008. Epigenetic events in mammalian germ-cell development: reprogramming and beyond. Nat Rev Genet. 9, 129–40.
Sattar, N., Greer, I. A., 2002. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? BMJ. 325, 157–60.
Schaller, F., Watrin, F., Sturny, R., Massacrier, A., Szepetowski, P., Muscatelli, F., 2010. A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. Hum Mol Genet. 19, 4895–905.
Schuster-Gossler, K., Simon-Chazottes, D., Guenet, J. L., Zachgo, J., Gossler, A., 1996. Gtl2lacZ, an insertional mutation on mouse chromosome 12 with parental origin-dependent phenotype. Mamm Genome. 7, 20–4.
Sedman, L., Padhukasahasram, B., Kelgo, P., Laan, M., 2008. Complex signatures of locus-specific selective pressures and gene conversion on Human Growth Hormone/ Chorionic Somatomammotropin genes. Hum Mutat. 29, 1181–93.
Seitz, H., Royo, H., Bortolin, M. L., Lin, S. P., Ferguson-Smith, A. C., Cavaille, J., 2004. A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. Genome Res. 14, 1741–8.

Sheikh-Hamad, D., Bick, R., Wu, G. Y., Christensen, B. M., Razeghi, P., Poindexter, B., Taegtmeyer, H., Wamsley, A., Padda, R., Entman, M., *et al.*, 2003. Stanniocalcin-1 is a naturally occurring L-channel inhibitor in cardiomyocytes: relevance to human heart failure. Am J Physiol Heart Circ Physiol. 285, H442–8.

Sherman, G. B., Wolfe, M. W., Farmerie, T. A., Clay, C. M., Threadgill, D. S., Sharp, D. C., Nilson, J. H., 1992. A single gene encodes the beta-subunits of equine luteinizing hormone and chorionic gonadotropin. Mol Endocrinol. 6, 951–9.

Shi, Q. J., Lei, Z. M., Rao, C. V., Lin, J., 1993. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. Endocrinology. 132, 1387–95.

Shoemaker, R., Deng, J., Wang, W., Zhang, K., 2010. Allele-specific methylation is prevalent and is contributed by CpG-SNPs in the human genome. Genome Res. 20, 883–9.

Simister, N. E., 2003. Placental transport of immunoglobulin G. Vaccine. 21, 3365–9.

Sitras, V., Fenton, C., Paulssen, R., Vartun, A., Acharya, G., 2012. Differences in Gene Expression between First and Third Trimester Human Placenta: A Microarray Study. PLoS One. 7, e33294.

Sitras, V., Paulssen, R., Leirvik, J., Vartun, A., Acharya, G., 2009a. Placental gene expression profile in intrauterine growth restriction due to placental insufficiency. Reprod Sci. 16, 701–11.

Sitras, V., Paulssen, R. H., Gronaas, H., Leirvik, J., Hanssen, T. A., Vartun, A., Acharya, G., 2009b. Differential placental gene expression in severe preeclampsia. Placenta. 30, 424–33.

Sivalingam, V. N., Duncan, W. C., Kirk, E., Shephard, L. A., Horne, A. W., 2011. Diagnosis and management of ectopic pregnancy. J Fam Plann Reprod Health Care. 37, 231–40.

Smallwood, A., Papageorghiou, A., Nicolaides, K., Alley, M. K., Jim, A., Nargund, G., Ojha, K., Campbell, S., Banerjee, S., 2003. Temporal regulation of the expression of syncytin (HERV-W), maternally imprinted PEG10, and SGCE in human placenta. Biol Reprod. 69, 286–93.

Soares, M. J., Muller, H., Orwig, K. E., Peters, T. J., Dai, G., 1998. The uteroplacental prolactin family and pregnancy. Biol Reprod. 58, 273–84.

Song, G., Bazer, F. W., Wagner, G. F., Spencer, T. E., 2006. Stanniocalcin (STC) in the endometrial glands of the ovine uterus: regulation by progesterone and placental hormones. Biol Reprod. 74, 913–22.

Song, G., Dunlap, K. A., Kim, J., Bailey, D. W., Spencer, T. E., Burghardt, R. C., Wagner, G. F., Johnson, G. A., Bazer, F. W., 2009. Stanniocalcin 1 is a luminal epithelial marker for implantation in pigs regulated by progesterone and estradiol. Endocrinology. 150, 936–45.

Springer, M. S., Murphy, W. J., Eizirik, E., O'Brien, S. J., 2003. Placental mammal diversification and the Cretaceous-Tertiary boundary. Proc Natl Acad Sci U S A. 100, 1056–61.

Srisuparp, S., Strakova, Z., Fazleabas, A. T., 2001. The role of chorionic gonadotropin (CG) in blastocyst implantation. Arch Med Res. 32, 627–634.

Steenman, M. J., Rainier, S., Dobry, C. J., Grundy, P., Horon, I. L., Feinberg, A. P., 1994. Loss of imprinting of IGF2 is linked to reduced expression and abnormal methylation of H19 in Wilms' tumour. Nat Genet. 7, 433–9.

Stenman, U. H., Alfthan, H., Hotakainen, K., 2004. Human chorionic gonadotropin in cancer. Clin Biochem. 37, 549–61.

Stenman, U. H., Tiitinen, A., Alfthan, H., Valmu, L., 2006. The classification, functions and clinical use of different isoforms of HCG. Hum Reprod Update. 12, 769–784.

Strahl, B. D., Allis, C. D., 2000. The language of covalent histone modifications. Nature. 403, 41–5.

Struwe, E., Berzl, G., Schild, R., Blessing, H., Drexel, L., Hauck, B., Tzschoppe, A., Weidinger, M., Sachs, M., Scheler, C., *et al.*, 2010. Microarray analysis of placental tissue in intrauterine growth restriction. Clin Endocrinol (Oxf). 72, 241–7.

Stumpo, D. J., Byrd, N. A., Phillips, R. S., Ghosh, S., Maronpot, R. R., Castranio, T., Meyers, E. N., Mishina, Y., Blackshear, P. J., 2004. Chorioallantoic fusion defects and embryonic lethality resulting from disruption of Zfp36L1, a gene encoding a CCCH tandem zinc finger protein of the Tristetraprolin family. Mol Cell Biol. 24, 6445–55.

Summers, K., Crespi, B., 2005. Cadherins in maternal-foetal interactions: red queen with a green beard? Proc Biol Sci. 272, 643–9.

Surani, M. A., Hayashi, K., Hajkova, P., 2007. Genetic and epigenetic regulators of pluripotency. Cell. 128, 747–62.

Than, N. G., Romero, R., Goodman, M., Weckle, A., Xing, J., Dong, Z., Xu, Y., Tarquini, F., Szilagyi, A., Gal, P., *et al.*, 2009. A primate subfamily of galectins expressed at the maternal-fetal interface that promote immune cell death. Proc Natl Acad Sci U S A. 106, 9731–6.

Tong, S., Wallace, E. M., Rombauts, L., 2006. Association between low day 16 hCG and miscarriage after proven cardiac activity. Obstet. Gynecol. 107, 300–304.

Toppings, M., Castro, C., Mills, P. H., Reinhart, B., Schatten, G., Ahrens, E. T., Chaillet, J. R., Trasler, J. M., 2008. Profound phenotypic variation among mice deficient in the maintenance of genomic imprints. Hum Reprod. 23, 807–18.

Tsai, S., Hardison, N. E., James, A. H., Motsinger-Reif, A. A., Bischoff, S. R., Thames, B. H., Piedrahita, J. A., 2011. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways. Placenta. 32, 175–82.

Tsui, D. W., Lam, Y. M., Lee, W. S., Leung, T. Y., Lau, T. K., Lau, E. T., Tang, M. H., Akolekar, R., Nicolaides, K. H., Chiu, R. W., *et al.*, 2010. Systematic identification of placental epigenetic signatures for the noninvasive prenatal detection of Edwards syndrome. PLoS One. 5, e15069.

Tycko, B., 2006. Imprinted genes in placental growth and obstetric disorders. Cytogenet Genome Res. 113, 271–8.

Varrault, A., Gueydan, C., Delalbre, A., Bellmann, A., Houssami, S., Aknin, C., Severac, D., Chotard, L., Kahli, M., Le Digarcher, A., *et al.*, 2006. Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell. 11, 711–22.

Verona, R. I., Mann, M. R., Bartolomei, M. S., 2003. Genomic imprinting: intricacies of epigenetic regulation in clusters. Annu Rev Cell Dev Biol. 19, 237–259.

Virgin, J. B., Silver, B. J., Thomason, A. R., Nilson, J. H., 1985. The gene for the beta subunit of bovine luteinizing hormone encodes a gonadotropin mRNA with an unusually short 5'-untranslated region. J Biol Chem. 260, 7072–7.

Wagschal, A., Feil, R., 2006. Genomic imprinting in the placenta. Cytogenet. Genome Res. 113, 90–98.

Wallace, C., Smyth, D. J., Maisuria-Armer, M., Walker, N. M., Todd, J. A., Clayton, D. G., 2010. The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. Nat Genet. 42, 68–71.

Wan, H., Versnel, M. A., Cheung, W. Y., Leenen, P. J., Khan, N. A., Benner, R., Kiekens, R. C., 2007. Chorionic gonadotropin can enhance innate immunity by stimulating macrophage function. J Leukoc Biol. 82, 926–33.

Weksberg, R., Shen, D. R., Fei, Y. L., Song, Q. L., Squire, J., 1993. Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. Nat Genet. 5, 143–50.

Wildman, D. E., 2011. Review: Toward an integrated evolutionary understanding of the mammalian placenta. Placenta. 32 Suppl 2, S142–5.

Wilkins, J. F., Ubeda, F., 2011. Diseases associated with genomic imprinting. Prog Mol Biol Transl Sci. 101, 401–45.

Winn, V. D., Haimov-Kochman, R., Paquet, A. C., Yang, Y. J., Madhusudhan, M. S., Gormley, M., Feng, K. T., Bernlohr, D. A., McDonagh, S., Pereira, L*., et al.*, 2007. Gene expression profiling of the human maternal-fetal interface reveals dramatic changes between midgestation and term. Endocrinology. 148, 1059–79.

Wolf, J. B., Hager, R., 2009. Selective abortion and the evolution of genomic imprinting. J Evol Biol. 22, 2519–2523.

Wood, A. J., Oakey, R. J., 2006. Genomic imprinting in mammals: emerging themes and established theories. PLoS Genet. 2, e147.

Xu, Y., Goodyer, C. G., Deal, C., Polychronakos, C., 1993. Functional polymorphism in the parental imprinting of the human IGF2R gene. Biochem Biophys Res Commun. 197, 747–754.

Yeung, B. H., Law, A. Y., Wong, C. K., 2012. Evolution and roles of stanniocalcin. Mol Cell Endocrinol. 349, 272–80.

Yu, L., Chen, M., Zhao, D., Yi, P., Lu, L., Han, J., Zheng, X., Zhou, Y., Li, L., 2009. The H19 gene imprinting in normal pregnancy and pre-eclampsia. Placenta. 30, 443–7.

Yuasa, S., Onizuka, T., Shimoji, K., Ohno, Y., Kageyama, T., Yoon, S. H., Egashira, T., Seki, T., Hashimoto, H., Nishiyama, T*., et al.*, 2010. Zac1 is an essential transcription factor for cardiac morphogenesis. Circ Res. 106, 1083–91.

Yue, L., Daikoku, T., Hou, X., Li, M., Wang, H., Nojima, H., Dey, S. K., Das, S. K., 2005. Cyclin G1 and cyclin G2 are expressed in the periimplantation mouse uterus in a cell-specific and progesterone-dependent manner: evidence for aberrant regulation with Hoxa-10 deficiency. Endocrinology. 146, 2424–33.

Yuen, R. K., Avila, L., Penaherrera, M. S., von Dadelszen, P., Lefebvre, L., Kobor, M. S., Robinson, W. P., 2009. Human placental-specific epipolymorphism and its association with adverse pregnancy outcomes. PLoS One. 4, e7389.

Yuen, R. K., Penaherrera, M. S., von Dadelszen, P., McFadden, D. E., Robinson, W. P., 2010. DNA methylation profiling of human placentas reveals promoter hypomethylation of multiple genes in early-onset preeclampsia. Eur J Hum Genet. 18, 1006–12.

Zhang, Y., Lang, Q., Li, J., Xie, F., Wan, B., Yu, L., 2010. Identification and characterization of human LYPD6, a new member of the Ly-6 superfamily. Mol Biol Rep. 37, 2055–62.

Zoricic, S., Maric, I., Bobinac, D., Vukicevic, S., 2003. Expression of bone morphogenetic proteins and cartilage-derived morphogenetic proteins during osteophyte formation in humans. J Anat. 202, 269–77.

Zygmunt, M., Herr, F., Munstedt, K., Lang, U., Liang, O. D., 2003. Angiogenesis and vasculogenesis in pregnancy. Eur J Obstet Gynecol Reprod Biol. 110 Suppl 1, S10–8.

8. SUMMARY IN ESTONIAN

Platsenta geeniekspressioon normaalses ja komplitseeritud raseduses

Platsenta on loote kest, mille kaudu on loode seotud ema organismiga. Raseduse jooksul vahendab platsenta loote ja ema vahelist suhtlust, reguleerides nii hapniku kui toit- ja jääkainete liikumist, sekreteerides erinevaid hormone ning kaitstes loodet ema immunoloogilise äratõukereaktsiooni ja välistegurite eest. Imetajates toimunud kiire liigisisese evolutsiooni tulemusena on platsentast kujunenud üks varieeruvama struktuuri ning geeniregulatsiooniga organeid. Platsentale on iseloomulikud duplikaatgeenide perekondade ja aktiivsete retrotransposonide rohkus ning teatud mikroRNA gruppide esinemine. Samuti on platsenta erakordne imprinditud geenide rikastatuse poolest. Imprinting on epigeneetiline mehhanism, mis tagab geenide monoalleelse avaldumise ainult ühelt vanemalt päritud geenialleelilt.

Platsenta ehitus, funktsioonid ning geeniregulatsioon muutuvad dünaamiliselt läbi raseduse, et tagada ema metabolismi kohanemine rasedusega ja optimaalne loote üsasisene areng. Inimese rasedus on jagatud kolmeks trimestriks. Esimesel trimestril toimub platsenta moodustumine, loote implantatsioon ning embrüonaalne areng. Teisel trimestri jooksul kujunevad detailselt välja loote organstruktuurid ning viimastele raseduskuudele on iseloomulik kiire loote kasv ning organite lõplik areng. Kuigi geneetiliselt on platsenta identne lootega, on loote ja platsenta geenide avaldumine erinev. Normaalseks raseduse kulgemiseks on tähtis platsenta geenide avaldumise korrektne ajaline ning ruumiline regulatsioon. Paraku ei ole inimese platsenta transkriptsioomi dünaamikat läbi kogu raseduse veel põhjalikult uuritud ning suurimad ebaselgused puudutavad raseduse teist trimestrit.

Üks tähtsamaid platsenta poolt toodetud rasedushormoone on inimese koorionгonadotropiin (human chorionic gonadotropin; hCG). Heterodimeerne glükoproteiin hCG koosneb α-subühikust ja vereseerumi hormooni taset määravast *β*-alaühikust. *β*-alaühikut kodeerib süntsüütsiotrofoblasti rakkudes avaldunud primaadispetsiifiline *CGB* geeniperekond. hCG süntees algab juba embrüos ning on vajalik embrüo implantatsioonil ja platsenta moodustumisel. Raseduse alguses stimuleerib hCG munasarja kollaskehas progresterooni tootmist, mis on vajalik raseduse säilimiseks esimestel rasedusnädalatel. Lisaks aitab hCG kaasa platsenta verevarustusele ning immuunotolerantsi kujunemisele ema ja loote vahel. Pärast embrüo pesastumist emaka limaskesta jõuab hCG esmakordselt ema verre ning on seal mõõdetav kogu raseduse jooksul. Seni pole *CGB* geenide ekspressiooni dünaamikat läbi normaalse raseduse veel uuritud. On teada, et normist oluliselt kõrgem või madalam hCG valgu tase seostub mitmete raseduskomplikatsioonidega. Näiteks on esimese trimestri korduvate raseduse katkemistega patsientidel hCG tase seerumis kriitiliselt madal ning samuti on alanenud *CGB* geenide transkriptsioon platsentas.

Normaalselt funktsioneeriv platsenta on eduka raseduse aluseks. Raseduse esimese poole tüsistused on enamasti seotud häiretega implanteerumisel ja platsenta moodustumisel, samuti immunoloogiliste ning verevarustuse probleemidega. Raseduse teises pooles avalduvad komplikatsioonid on seotud ema ja loote metaboolsete häiretega. Patoloogiate kujunemise molekulaarsed mehhanismid ning geeniekspressiooni dünaamika on seni põhjalikult kaardistamata. Sellest tulenevalt on hiljuti järjest enam tähelepanu kogumas komplitseeritud rasedustest pärineva platsenta geeniekspressiooni uuringud. Uuringute eesmärgiks on teha kindlaks molekulaarsed mehhanismid, mis on seotud raseduspatoloogiate kujunemisega, kuid samuti uute mitteinvasiivsete biomarkerite ning ravimisihtmärkide tuvastamine.

Käesoleva doktoritöö kirjanduse ülevaade käsitleb platsenta evolutsiooni imetajates, kirjeldab platsenta omadusi ning molekulaarset evolutsiooni primaatides ja inimeses ning tutvustab lähemalt primaatide platsentas avaldunud kooriongonadotropiini hormooni ja selle β-alaühikut kodeerivaid geene. Järgmisena tutvustatakse inimese platsenta geeniekspressiooni alaseid uuringuid ning antakse ülevaade platsenta geenide epigeneetilisest regulatsioonist.

Uurimustöö eksperimentaalse osa eesmärkideks on (a) võrdlevalt uurida platsenta geenide avaldumist normaalse ja komplitseeritud raseduse korral ning (b) tuvastada geenid, mille avaldumine ning funktsioon on rasedustüsistuste platsentas häiritud. Kitsamalt keskendub töö kahe suurema alateema uurimisele:

1) Platsenta-spetsiifiliste hCG β-alaühikut kodeerivate geenide ekspressiooni analüüs, et
   - tuvastada *CGB* geenide avaldumine normaalse raseduse platsentas I, II ja III trimesteril ning võrdlevalt I trimestri raseduskomplikatsioonide platsentades (korduv iseeneslik raseduse katkemine, emakaväline rasedus, moolrasedus);
   - uurida kõgeima transkriptsiooni aktiivsusega *hCGβ* geenide, *CGB5* ja *CGB8*, ekspressiooni vaigistamist DNA metüleerimise teel korduvate raseduse katkemiste platsentades.
2) Platsenta transkriptoomi kirjeldamine raseduse I ja II trimestritel mikrokiipide abil, et
   - määrata kindlaks geenid, mis on spetsiifiliselt avaldunud inimese platsentas raseduse II trimestril;
   - uurida, kas II trimestri platsentas avaldunud geenide ebatüüpiline ekspressioon raseduse III trimestril on seotud ema ja loote raseduspatoloogiatega (preeklampsia, rasedusaegne diabeet, loote kasvuhäired);
   - määrata kõige olulisemate rasedustüsistustega seotud geenide valgu avaldumise tase normaalse ja komplitseeritud raseduste korral ning hinnata nende võimalikku edasist rakendamist biomarkerina.

Uurimistöö peamised tulemused on:

1) *CGB* geenide transkriptsioon normaalse raseduse platsentades on kõrgeim raseduse esimesel trimestril, langeb oluliselt teiseks trimestriks ning tõuseb taas kolmandal trimestril. Nii nagu hCG hormooni tase, varieerub ka *CGB* geenide transkriptsioon suuresti indiviidide ja kliiniliste gruppide vahel.
2) Korduva raseduse katkemise platsentades on *CGB* geenide transkriptsioon oluliselt alanenud võrreldes esimese trimestri normaalse rasedusega (P=0.03).
3) *CGB5* ja *CGB8* geenid on normaalse raseduse platsentades avaldunud võrdselt mõlemalt vanemalt päritud geenialleelidelt.
4) Kahes korduva raseduse katkemise platsentas ja ühes ühes esimese trimestri raseduse katkestuse platsentas oli *CGB5* geen transkribeeritud vaid emalt päritud alleelidelt. *CGB5* promootorala DNA metülatsiooni analüüs näitas, et *CGB5* geeni promootor oli nendes kudedes hemimetüleeritud. Seega võib *CGB5* geeni isalt päritud alleelide vaigistamine toimuda promootorala metüüleerimise tulemusel.
5) Normaalse raseduse esimese ja teise trimestri 5–18 rasedusnädalatel kogutud platsentade transkriptoomi analüüs tuvastas 154 oluliselt suurenenud või alanenud avaldumisega geeni (ANOVA; FDR korrigeeritud P<0.1).
6) Mikrokiibi tulemuste RT-qPCR replikatsioon ja validatsioon 24 geeni korral kinnitas üldjoontes mikrokiibi analüüsi tulemusi ning tõi välja 16 statistiliselt olulise signaaliga geeni. Kümne geeni ekspressioon oli normaalse raseduse jooksul oluliselt kõrgem II trimestri platsentas võrreldes I ja III trimestriga (*BMP5*, *CCNG2*, *CDH11*, *FST*, *GATM*, *GPR183*, *ITGBL1*, *PLAGL1*, *SLC16A10*, *STC1*). Nelja uuritud geeni puhul suurenes ekspressioon oluliselt teiseks trimestriks ning jäi saavutatud tasemele kuni raseduse lõpuni (*LYPD6*, *MEG3*, *NRCAM*, *ZFP36L1*). Kahe geeni ekspressioon suurenes pidevalt läbi kogu raseduse (*NEDD9*, *NR3C1*).
7) Leitud geenide ekspressioon on häiritud raseduskomplikatsioonidega platsentades, mis on seotud ema metabolismi häirete (preeklampsia, rasedusaegne diabeet) ja loote kasvuanomaaliatega (loote kasv liiga väike/suur sõltuvalt gestatsiooni ajast).
8) Valgu STC1 tase ema veres on oluliselt kõrgem preeklampsia, loote kasvupeetuse ja ema rasedusaegse diabeedi korral (P<0.035). Kõrgeim plasma STC1 on iseloomulik kompleksanomaaliale, milles loote kasvupeetus on kombineeritud ema preeklampsiaga (P=4.8x10^{-4}). Edasised uuringud näitavad, kas STC1 glükoproteiini on võimalik kasutada biomarkerina.
9) Kahe uuritava valgu (LYPD6 ja CCNG2) ekspressioon platsentas on oluliselt suurenenud preeklampsia ja rasedusaegse diabeediga komplitseeritud rasedustes. Lisaks demonstreeris käesolev töö esmakordselt LYPD6 valgu ekspresiooni platsenta süntsüütsiotrofoblastis.
10) Kirjanduse analüüs näitas, et geenid, mis on raseduse keskpaigas platsentas kõrgelt avaldunud, võivad lisaks raseduspatoloogiatele olla seotud ka mitmete täiskasvanuea krooniliste haigustega, nagu näiteks artriidi, kroonilise neerupuudulikkuse ja diabeediga.

Kokkuvõttes käsitleb antud doktoriväitekiri platsenta geeniekspressiooni kahest vaatenurgast. Esiteks uuritakse hüpoteesipõhiselt *CGB* geenide avaldumist platsentas ning teiseks viiakse läbi globaalne platsenta transkriptoomi analüüs ning selle validatsioon. Uurimus keskendub normaalse ja komplitseeritud raseduse võrdlusele.

Üks töö olulisematest leidudest on *CGB* geenide avaldumismuster normaalses raseduses ning häiritud avaldumine rasedustüsistuste korral. See leid kinnitab *CGB* geenide rolli embrüo implantatsioonil, platsentatsioonil ning varajase raseduse säilitamisel.

Käesolevas töös uuriti esmakordselt platsenta geeniekspressiooni dünaamikat inimese raseduse esimese ja teise trimestri jooksul. Raseduse esimese poole transkriptoomi analüüs tuvastas mitmed geenid, mis on kõrgelt ekspreseeritud teise trimestri platsentas. Nende geenide häiritud avaldumine on iseloomulik kolmanda trimestri rasedusanomaaliatele, kuid samuti kroonilistele täiskasvanuea haigustele.

Kuna rahvastiku kasv ja vananev elanikkond avaldavad ühiskonnale järjest suuremat mõju, on tööea pikendamine ja haiguste ennetamine üks tuleviku teaduse ja meditsiini väljakutsetest. Platsenta funktsioon mõjutab ema ja loote heaolu nii raseduse ajal kui ka edasises elus. Seega omab platsenta geeniregulatsiooni mõistmine normaalses ja komplitseeritud raseduses suurt kliinilist ja sotsiaalset tähtsust.

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