DISSERTATIONES
BIOLOGICAE
UNIVERSITATIS
TARTUENSIS
445

MARIO REIMAN

Placental transcriptome in normal and complicated pregnancies

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
445

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
445

MARIO REIMAN

Placental transcriptome in normal and complicated pregnancies

UNIVERSITY OF TARTU
Press
1632

Institute of Molecular and Cell Biology, University of Tartu, Estonia

This dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Gene Technology on December 4, 2024 by the Council of the Institute of Molecular Cell Biology, University of Tartu.

Supervisor:	Maris Laan, Ph.D., Professor of Human Genetics, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Estonia
Reviewer:	Maido Remm, Ph.D., Professor of Bioinformatics, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu
Opponent:	prof. Hannele Laivuori, Helsinki University
Commencement:	Room No. 105, 23B Riia St., Tartu, on January 21, 2025, at 10.15 pm.

This research was funded by grants from the Estonian Research Council (IUT34-12, PRG1021), the European Union through the European Regional Development Fund (project HAPPY PREGNANCY, 3.2.0701.12-0047) and PER ASPERA (2014-2020.4.01-0027; grant holder: University of Tartu) as well as personal stipends from Archimedes Foundation, Graduate school in Biomedicine and Biotechnology and COST Association.

ISSN 1024-6479 (print)
ISBN 978-9916-27-719-5 (print)
ISSN 2806-2140 (pdf)
ISBN 978-9916-27-720-1 (pdf)

University of Tartu Press
www.tyk.ee

“If I have seen further, it is by standing on the shoulders of Giants.”

Sir Isaac Newton

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by Roman numerals (Ref. I to Ref. IV):

I. **Mario Reiman**, Maris Laan, Kristiina Rull, and Siim Sõber. 2017. "**Effects of RNA Integrity on Transcript Quantification by Total RNA Sequencing of Clinically Collected Human Placental Samples.**" FASEB Journal 31 (8): 3298–3308. https://doi.org/10.1096/fj.201601031RR.

II. Sõber, Siim, **Mario Reiman,** Triin Kikas, Kristiina Rull, Rain Inno, Pille Vaas, Pille Teesalu, Jesus M.Lopez Lopez Marti, Pirkko Mattila, and Maris Laan. 2015. "**Extensive Shift in Placental Transcriptome Profile in Preeclampsia and Placental Origin of Adverse Pregnancy Outcomes.**" Scientific Reports 5 (1): 13336. https://doi.org/10.1038/srep13336.

III. Sõber, Siim, Kristiina Rull, **Mario Reiman**, Piret Ilisson, Pirkko Mattila, and Maris Laan. 2016. "**RNA Sequencing of Chorionic Villi from Recurrent Pregnancy Loss Patients Reveals Impaired Function of Basic Nuclear and Cellular Machinery.**" Scientific Reports 6 (1): 38439. https://doi.org/10.1038/srep38439.

IV. Diana Pilvar#, **Mario Reiman#**, Arno Pilvar, and Maris Laan. 2019. "**Parent-of-Origin-Specific Allelic Expression in the Human Placenta Is Limited to Established Imprinted Loci and It Is Stably Maintained across Pregnancy.**" Clinical Epigenetics 11 (1): 1–14. https://doi.org/10.1186/S13148-019-0692-3.
first shared authors

My contributions to the listed publications were as follows:

Ref. I Conceived and designed the research, analyzed and interpreted the data, wrote the first draft of the manuscript and critically reviewed the improved version of the paper. Finalized manuscript.

Ref. II–III Optimized the laboratory protocol, and performed all placental RNA extractions; performed experiments for the RNA-Seq library preparation. Participated in the data analysis and interpretation, discussed and interpreted the results and approved the final manuscript.

Ref. IV Contributed to the bioinformatics analysis and interpreted the outcome data, participated in writing the manuscript, and critically reviewed and commented on the paper.

LIST OF ABBREVIATIONS

AIM1	absent in melanoma 1 protein
BMI	Body mass index
BP	biological process
C19MC	chromosome 19 miRNA cluster
CC	cellular component
cDNA	complementary DNA
CGB	chorionic gonadotropin
CI	confidence interval
CPXM2	carboxypeptidase X, M14 family member 2
DCAF10	DDB1 and CUL4 associated factor 10
DE	differential expression
DEG	differentially expressed genes
DLK1	delta like non-canonical notch ligand 1
DNMT1	DNA methyltransferase 1
EDTA	ethylenediamine tetra-acetic acid
ENG	endoglin
ESRRG	estrogen related receptor gamma
FFPE	formalin-fixed paraffin-embedded
FLT	fms related receptor tyrosine kinase
FPKM	fragments per kilobase of transcript per million mapped reads
g.d.	gestational day
g.w.	gestational week
GC%	guanine/cytosine content
GCM1	glial cells missing transcription factor 1
GD	gestational diabetes mellitus
GH	gestational hypertension
GO	gene ontology
GRB10	growth factor receptor bound protein 10
GRHL1	grainyhead like transcription factor 1
GRINA	glutamate ionotropic receptor NMDA type subunit associated protein 1
GTEx	genotype-tissue expression project
H19	H19 imprinted maternally expressed transcript
HIST1H2AE	H2A clustered histone 8
HIST1H4F	H4 clustered histone 6

HIST2H2AC	H2A clustered histone 20
HOOK2	hook microtubule tethering protein 2
hPL	human placental lactogen
HTRA4	HtrA serine peptidase 4
IGF2	insulin like growth factor 2
IGFBP	insulin like growth factor binding protein
IUGR	Intrauterine growth restriction
kb	kilobase
KLHDC10	kelch domain containing 10
LEP	leptin
LGA	large-for-gestational-age
lncRNA	long non-coding RNA
LO-PE	late-onset preeclampsia
MAF	minor allele frequency
Mat	maternal
MCCC1	methylcrotonyl-CoA carboxylase subunit 1
MEG3	maternally expressed 3
MEST	mesoderm specific transcript
MF	molecular function
MHC	major histocompatibility complex
miRNA	microRNA
MKRN3	makorin ring finger protein 3
MMP	matrix metallopeptidase
mRNA	messenger RNA
n.a.	not available
n.d.	no date
NAA60	N-alpha-acetyltransferase 60, NatF catalytic subunit
ncRNA	noncoding RNA
NLRP2	NLR family pyrin domain containing 2
NOTCH2	notch receptor 2
NRIP1	nuclear receptor interacting protein 1
NUDT12	nudix hydrolase 12
Pat	paternal
P_{corr}	Bonferroni corrected P value
PCR	polymerase chain reaction
PE	preeclampsia

PEG10	paternally expressed 10
pEIF2α	eukaryotic initiation factor-2α phosphorylation
PERK	protein kinase R-like endoplasmic reticulum kinase
PGH	placental growth hormone
PHLDA2	pleckstrin homology like domain family A member 2
PIGT	phosphatidylinositol glycan anchor biosynthesis class T
PLAGL1	PLAG1 like zinc finger 1
PLEKHG4B	pleckstrin homology and RhoGEF domain containing G4B
PPE	predominantly placenta-expressed
PTB	preterm birth
REAC	reactome pathway database
REPROMETA	REPROgrammed fetal and/or maternal METAbolism
RHOBTB3	Rho related BTB domain containing 3
RIN	RNA integrity number
RNA-Seq	RNA sequencing
RPL	recurrent pregnancy loss
RTL1	retrotransposon gag like 1
RT-qPCR	quantitative reverse transcription PCR
SASH1	SAM and SH3 domain containing 1
SD	standard deviation
SGA	small-for-gestational-age
SH3PXD2A	SH3 and PX domains 2A
SNP	single nucleotide polymorphism
TERM NORM	normal term pregnancies
UTR	untranslated region
ZDBF2	zinc finger DBF-type containing 2
ZFAT	zinc finger and AT-hook domain containing

INTRODUCTION

Pregnancy is the term used to describe the period during which a developing embryo or fetus grows and develops within a woman's uterus. It is a complex process that typically lasts around 40 weeks, divided into three trimesters. During pregnancy, the mother's body undergoes numerous changes to support the developing baby, including the formation of a placenta. The placenta plays a crucial role during pregnancy as it is an organ that develops in the uterus and provides oxygen and nutrients to the growing baby while also removing waste products from the baby's blood. The placenta forms from the same cells that form the embryo, and it is closely connected to the baby through the umbilical cord. In summary, pregnancy and the placenta are closely related as the placenta plays a vital role in supporting the development and well-being of the growing baby during pregnancy.

However, not every pregnancy progresses without complications. Statistics show that approximately 7–21% of pregnancies are affected by hypertensive disorders (Umesawa & Kobashi, 2016), and 1–5% of couples experience recurrent pregnancy loss (Jauniaux et al., 2006; Regan et al., 2023). Previous research has convincingly established a link between pregnancy complications and aberrant placental gene expression. Therefore, studying the placental transcriptome could provide valuable insights for diagnosing, preventing, and treating these conditions.

The principal aim of the thesis was to develop the experimental and analytical pipeline to generate and profile placental transcriptome datasets. We assembled, at the time, the biggest dataset of placental RNA-Seq transcriptomes that included complicated and uncomplicated pregnancies from across all three trimesters. As part of the quality assurance process, I clarified to what extent RNA degradation, an extensive process after placental delivery, might affect the results of such experiments and data analysis. My study analyzed what genes and pathways are differentially expressed in the placentas of early and late pregnancy complications, and examined parental allelic expression of placental genes in various gestational outcome scenarios.

1. LITERATURE REVIEW

1 1. Human pregnancy

Human pregnancy or gestation is a complex biological process during which a fetus develops inside a woman's uterus. With fertilization, a single cell called a zygote is formed from the fusion of the oocyte and the sperm. The zygote then undergoes a series of cell divisions to form a blastocyst, and a week later implants itself into the uterine lining (Hill, 2010). There it continues its development, forming both the fetal side of the placenta and the embryo proper that will be programmed to become a fetus (Hyun et al., 2020). Placenta facilitates the development of the fetus by carrying oxygen and nutrients from the mother to the fetus and waste materials from the fetus to the mother. It also produces several hormones that help maintain pregnancy (Fowden et al., 2015).

1.1.1. Course of normal pregnancy

The duration of the human pregnancy is typically 40 gestational weeks (g.w.) from the start of the last menstrual period before conception to childbirth (Jukic et al., 2013; Tanbo et al., 2018). Human pregnancy is conventionally divided into three trimesters: 1st trimester – until the 12th gestational week, 2nd trimester – until the 27th gestational week, and onwards from the 28th, the third trimester.

During the first trimester, from fertilization to 12 weeks gestation, the zygote undergoes multiple cell divisions to form a blastocyst. The blastocyst then undergoes implantation into the endometrium, and the embryonic disk begins to differentiate into the three germ layers. By Carnegie stage 7 (around day 17–18 post-fertilization (Hill, 2010), the embryonic disk has formed, and the primitive streak appears, signalling the beginning of gastrulation. By Carnegie stage 13 (around week 7), the fetus has a distinct human-like appearance, and by Carnegie stage 23 (around week 10–12), all major organ systems are present. By the end of the first trimester, the embryo has developed most of its major organs and structures, including the neural tube, heart, limbs, and gastrointestinal tract. At that time in humans, the embryo is no longer called the embryo but a fetus.

During the second trimester, which spans from 13 to 27 weeks gestation, the fetus is fully formed, mostly just gains weight, and refines its organ systems (Figure 1). The fetus gains substantial weight and length during this trimester, and its movements become more coordinated (Hill, 2010). Over the course of the second trimester, the placenta continues to develop and grow. The chorionic villi increase in number and size, and the placenta becomes more efficient at transferring oxygen and nutrients to the fetus and removing waste products (Gude et al., 2004).

During the third trimester, which spans from 28 weeks until birth, the fetus continues to grow and mature. During this trimester, the fetus undergoes significant brain development, and its lungs mature in preparation for breathing air

after birth. The placenta reaches its maximum size and efficiency (Figure 1). However, as the fetus grows larger, the placenta may begin to show signs of ageing and reduced function (Gude et al., 2004). In some cases, this can lead to complications such as preeclampsia or fetal growth restriction. By the end of the third trimester, the fetus is considered full-term and is ready for birth.

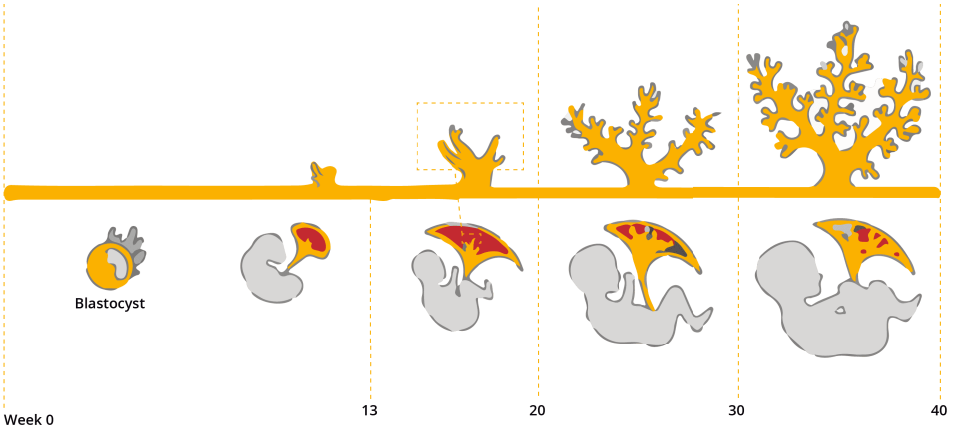

Figure 1: development of fetus and placenta across gestational weeks. Adapted from https://www.vedantu.com/biology/placenta (*Placenta – Structure, Development, Functions and Facts*, n.d.).

1.1.2. Placenta as a unique organ

The placenta is a crucial organ during pregnancy, serving as the interface between the mother and fetus (Gheorghe et al., 2010). Interestingly, the placenta has a distinct genome different from the mother's. This means that the placenta, while shared between the mother and fetus, has its own unique genetic identity (X. Wang et al., 2013).

This unique genetic identity can cause the placenta to behave similarly to cancer (Lala et al., 2021). The placenta must grow and develop rapidly to support the growing fetus, but it must also be regulated to prevent uncontrolled growth (Sandovici et al., 2022).

The tug-of-war between the maternal and paternal genomes in the placenta contributes to this delicate balance. The paternal genes tend to promote placental growth, while the maternal genes work to restrict it. This "battle of the sexes" within the placenta is an essential part of its development and function (Sandovici et al., 2022).

Understanding the complex interplay between the mother, fetus, and placenta is crucial for understanding pregnancy and fetal development (Sood et al., 2006). The unique genomic identity of the placenta and its cancer-like behaviour are key aspects of this dynamic system (Lala et al., 2021; Sandovici et al., 2022; X. Wang et al., 2013).

1.2. Placenta and pregnancy complications

1.2.1. Common pregnancy complications

Placental complications during pregnancy can have serious effects on both the mother and the fetus. Issues with implantation, early development, or functional capacities in later pregnancy due to fetal growth can increase the risk of complications. Some of the most common pregnancy complications include preeclampsia (PE), gestational hypertension (GH), gestational diabetes mellitus (GD), recurrent pregnancy loss (RPL), Intrauterine growth restriction (IUGR), small-for-gestational-age (SGA), large-for-gestational-age (LGA), and preterm birth (PTB) (Table 1).

Table 1: Prevalence and definitions of common pregnancy complications.

Pregnancy complication	Prevalence	Definition	References
PTB	4.1–19.1%	defined as delivery before 37 completed weeks of gestation	Ahmed et al., 2023; Torchin & Ancel, 2016
GH	1.8–4.4%	defined as a systolic BP >140 mmHg or a diastolic BP >90 mmHg, with no proteinuria, developed after 20 g.w.	Lai et al., 2017; Umesawa & Kobashi, 2016
PE	0.2–9.2%	defined as gestational hypertension with organ dysfunction, e.g. proteinuria, transaminitis, congestive heart failure, IUGR or neurologic symptoms.	Lai et al., 2017; Umesawa & Kobashi, 2016
IUGR	10–15%	Birth weight below the tenth percentile with optional evidence of abnormal cord blood flow	Armengaud et al., 2021
SGA	10%	birth weight of less than 10^{th} percentile for gestational age	Moreira & Méio, 2022
LGA	10%	birth weight >90^{th} percentile for gestational age	(Shu et al., 2023)
GD	4–35%	defined as glucose intolerance that is first diagnosed in pregnancy	Eades et al., 2017; Mazumder et al., 2022
RPL	1–5%	three consecutive pregnancy losses, including non visualised ones	Eleje et al., 2023; Jauniaux et al., 2006; Regan et al., 2023

PTB – preterm birth; PE – preeclampsia; GH – gestational hypertension; GD – gestational diabetes mellitus; RPL – recurrent pregnancy loss; IUGR – Intrauterine growth restriction; SGA – small for gestational age; LGA – large for gestational age

1.2.2. Preeclampsia

Preeclampsia is one of the most well-known hypertensive disorders of pregnancy (HDP). Other HDP include chronic hypertension, gestational hypertension, chronic hypertension with superimposed PE and eclampsia (Akre et al., 2022). Hypertension in pregnancy is defined as a systolic pressure of 140 mmHg and/or a diastolic pressure of 90 mmHg. Preeclampsia is defined as gestational hypertension with proteinuria >0.3 g in a 24-hour urine collection or as organ dysfunction defined by a platelet count <100,000/mm3, a creatinine level >1.1 mg/dL, transaminitis, congestive heart failure, IUGR or neurologic symptoms (Lai et al., 2017). Definitive therapy is delivery, but conservative management may be pursued in selected cases, particularly if the condition occurs early in gestation (thus enabling maximal time for the fetus to mature). However, preeclampsia is a precursor to eclampsia, and if the mother develops tonic-clonic seizures, it is considered an eclamptic episode. Eclampsia and preeclampsia during pregnancy are known to cause morbidity and even death in both the mother and fetus if not adequately diagnosed (Akre et al., 2022).

While the aetiology of preeclampsia is not fully understood, it is recognized that the placenta is central to this condition's development. A two stage model of preeclampsia has been proposed, where a poorly perfused placenta (Stage 1) produces factor(s) leading to the clinical manifestations of preeclampsia (Stage 2). Stage 1 might also induce the development of other confounding pregnancy complications, such as intrauterine growth restriction (Roberts & Escudero, 2012; Roberts & Hubel, 2009).

Risk factors that increase the odds of developing PE are as follows (Duley, 2009):

- Chronic high blood pressure or kidney disease before pregnancy
- High blood pressure or preeclampsia in an earlier pregnancy
- Obesity. Women who are overweight or obese are also more likely to have preeclampsia in more than one pregnancy.
- Diabetes, both type 1 and 2 diabetes increase the risk 2- to 4-fold (Weissgerber & Mudd, 2015).
- Age. Women older than 40 are at higher risk.
- Multiple gestation (being pregnant with more than one fetus)
- African American ethnicity.
- The previous occurrence of PE increases the relative risk by 10- to 25-fold (Boyd et al., 2013).
- Family history of preeclampsia increases the risk from 1.24- to 2.3-fold (Boyd et al., 2013; Carr et al., 2005).

1.2.3. Recurrent pregnancy loss

Miscarriage is a common complication of pregnancy, occurring in about 13% of clinically confirmed pregnancies before 22 weeks of gestation (Bricker & Farquharson, 2002). In fact, even before implantation, about 30% of human conceptions are lost and another 30% are detected only as 'biochemical pregnancies' that do not develop further (Larsen et al., 2013). The definition of recurrent pregnancy loss ranges from two miscarriages according to the American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE), to three consecutive pregnancy losses as defined by the Royal College of Obstetricians and Gynaecologists (RCOG) (Youssef et al., 2019). In the context of this thesis, we are using the RCOG definition (Regan et al., 2023). By that definition RPL is a condition that affects about 1–5% of couples, and that frequency is far higher than what we would otherwise expect from sporadic occurences of miscarriage. While most sporadic cases of pregnancy loss are due to chromosomal abnormalities, RPL can have various causes, including chromosomal aberrations (10%) (Pal et al., 2018). In addition, there are several female-related causes that can lead to RPL. These factors include anatomic abnormalities like uterine malformations, fibroids, and endometrial polyps (10–20%), and endocrine disorders such as subclinical hypo- or hyperthyroidism, hyperprolactinemia, and menstrual disorders (10–20%). Parental immunological factors such as antiphospholipid syndrome of the female partner are considered as critical contributors to RPL in 10–20% of cases. Infections in the genital tract of either partner are responsible for 0.5–5% of RPL cases (Kasak et al., 2018). However, in 20–50% of cases, no clear cause can be identified, making RPL an unknown condition with immediate and long-term consequences for affected families. A study from the Danish Recurrent Pregnancy Loss Clinic found that even after being referred to a specialized clinic, 33% of RPL couples remain childless after five years (Lund et al., 2012). Epidemiological studies have shown that subsequent pregnancies after a series of miscarriages have an increased risk of preterm birth, stillbirth, preeclampsia, and fetal growth restriction (Petriglia et al., 2015; Van Oppenraaij et al., 2009). Therefore, it is essential to uncover new mechanisms behind RPL to develop biomarkers and treatments to benefit affected patients. Studying gene expression profiles and biological pathways involved in failed pregnancy could potentially identify novel biomarkers or therapeutic targets applicable in clinical conditions for the benefit of RPL patients.

1.3. Placental transcriptome

The placental transcriptome refers to the complete set of RNA molecules that are expressed in the placenta. Recent advances in high-throughput sequencing technology have made it possible to analyze the placental transcriptome in unprecedented detail, providing insights into the complex molecular mechanisms that underlie placental development and function. Despite that, the placenta is often excluded from large-scale transcriptomics studies, such as the Genotype-Tissue Expression (GTEx) project (Aguet et al., 2020).

1.3.1. Special features of placental transcriptome

While the primary function of the placenta remains the same across mammalian species, there is a significant degree of anatomical variation observed in terms of shape, level of intimacy between fetal and maternal tissues, and degree of interdigitation between these tissues. Additionally, mammals exhibit a range of differences in the number and size of their offspring, as well as the length of gestation, which are often linked to specific features of placental morphology (Armstrong et al., 2017). The human placenta exhibits a unique genomic architecture with an unexpectedly high mutation burden (Kasak et al., 2015, 2017).

One area of active research in the field of placental transcriptomics is the identification of predominantly placenta-expressed (PPE) genes. These are genes that are uniquely expressed in the placenta and not transcribed at all or only at low levels in other tissues. Transcriptome profiling has been a widely utilized method of choice to identify placenta-specific genes (Szilagyi et al., 2020; Yong & Chan, 2020).

Remarkably, separate observations have noted that many PPE genes are encoded by primate-specific gene clusters (e.g., chorionic gonadotropins (*CGB*s), galectins, microRNAs), suggesting that the evolution of these clusters and their trophoblastic expression might have strongly impacted the complex regulation of pregnancy in humans (Inno et al., 2021; Nagirnaja et al., 2010). Preeclampsia is associated with dysregulated expression or function of many PPE genes (e.g., *PGH*, *hPL*, *LEP* (Männik et al., 2012; Ratnik et al., 2022)), transcription factors (e.g., GCM1, ESRRG (Jeyarajah et al., 2022; Zou et al., 2021)), and signalling and kinase pathways (e.g., Wnt/β-catenin, PERK-pEIF2α (Lian et al., 2011; Rana et al., 2019)), emphasizing the importance of placenta-specific genes to successful pregnancy (Szilagyi et al., 2020).

MicroRNAs (miRNAs) are small non-coding RNA molecules that play important roles in gene regulation. More than 1000 mature miRNAs are identified in human genomes, among which more than 600 miRNAs have been found in the human placenta (Xu et al., 2021). The chromosome 19 miRNA cluster (C19MC) is known as placental-specific because it is mainly expressed in the placenta among all miRNAs. C19MC cluster is the most significant miRNA gene cluster identified in the human genome to date, which spans about 100 kb on chromosome 19q13.4 and includes around 54 miRNAs. C19MC family is solely found

in primates. Interestingly, C19MC is controlled by genomic imprinting where only the paternally-inherited allele is expressed. Pregnancy complications such as pregnancy loss, preeclampsia, intrauterine growth restriction, gestational diabetes mellitus (GD), obesity, and preterm birth have been linked with miRNAs in the placenta (Hu & Zhang, 2019; Inno et al., 2021). Additionally, placenta miRNAs have been helpful in distinguishing between normal pregnancies and pregnancy-related illnesses, highlighting their significance in the pathogenesis of these diseases (Addo et al., 2020).

While there are several genes that are placenta-specific, the number of genes that are not uniquely expressed in that specific tissue is also surprisingly large. Compared to all other organs studied in the GTEx project, the human placenta has a unique transcriptome architecture, with more than 80% of the organs lacking even one uniquely depleted transcript. The liver had the second-highest number of uniquely depleted transcripts, with 26 being depleted or absent. In contrast, the transcriptome of the term placenta exhibited 762 depleted or absent transcripts. Some of the absent transcripts relate to unique qualities of placental function. For example, because the placenta has no innervation, multiple transcripts involved in forming elements of the nervous system are not expressed. Additionally, major histocompatibility complex (MHC) antigens which play a role in the alloimmune placenta is an another affected pathway. However, many of the affected genes are associated with mitochondrial function (S. Gong et al., 2023).

1.3.2. Genomic imprinting in placenta

The monoallelic expression of certain genes in mammals is governed by an epigenetic process known as genomic imprinting. In mammals, imprinted genes are known to regulate placental development and fetal growth and are believed to have coevolved with placentation (Angiolini et al., 2021). Most mammals express their autosomal genes codominantly from the 2 parental chromosomes. However, at some loci, the allele inherited from one parent is suppressed through an epigenetic mechanism that results in imprinted monoallelic parent-of-origin-specific expression. This monoallelic expression is achieved by epigenetic asymmetries between parental alleles that include differential DNA methylation, histone modifications, antisense noncoding RNA (ncRNA) – mediated silencing, and long-range chromatin interactions (Monk, 2015). The number of imprinted genes in humans is thought to be about 165 (Tucci et al., 2019); most are expressed in the placenta and at least 27 of those are expressed only in the placenta (Monk, 2015). Imprinted genes have been shown to be related to fetal growth (SGA, LGA, IUGR), preeclampsia, premature birth, and the success rate of assisted reproductive technology procedures (Angiolini et al., 2021; X. Gong et al., 2024; Monk, 2015).

1.4. Typical workflow of human placental transcriptome research

The study of the human placental transcriptome has evolved significantly over the past two decades, driven by technological advancements and a deepening understanding of placental biology. This section outlines the typical workflow for contemporary human placental transcriptome research, from sample collection to data interpretation.

1.4.1. Sample collection and RNA extraction

The first step in placental transcriptome research is the collection and preparation of placental samples. Placental tissue is typically obtained immediately after delivery, with care taken to minimize the time between delivery and sample processing to preserve RNA integrity (Burton et al., 2014). Cox et al. (2015) emphasize the importance of standardized collection protocols, noting that factors such as mode of delivery, gestational age, and maternal conditions can significantly impact gene expression profiles (Cox et al., 2015).

After collection, total RNA is extracted from the placental tissue, usually using TRIzol and/or spin column-based commercial kits (Cox et al., 2015). The extracted RNA undergoes rigorous quality control measures, typically including quantification using spectrophotometric methods and assessment of RNA integrity, often using the RNA Integrity Number (RIN) generated by the Bioanalyzer system (Schroeder et al., 2006). While microarrays can accept whole RNA as an input material, in RNA-Seq methods rRNA is almost always removed from the RNA solution. This process is most commonly carried out using poly-A-tailed mRNA selection or ribodepletion. Poly-A selection offers a more cost-effective solution for gene expression studies focusing on the protein-coding fraction of transcriptome, whereas ribodepletion covers a wider selection of transcriptome and works efficiently with degraded RNA (Kumar et al., 2017; Zhao et al., 2018). High-quality RNA is essential for reliable transcriptome analysis. How much and what kinds of transcripts are more affected are dependent on the exact methodology of sample preparation and quantification (Duan et al., 2013; Feng et al., 2015; Gallego Romero et al., 2014; Reiman et al., 2017; Sharova et al., 2009). Before 2013, when our group started conducting our RNA-seq library preparation, the scientific literature had established that RNA integrity significantly affected gene expression quantification in microarray experiments. However, no articles had been published regarding its effects on RNA-seq results (Reiman et al., 2017).

1.4.2. Transcriptome profiling: from microarrays to RNA sequencing

Historically, microarray technology was the primary tool for transcriptome profiling in placental research. Microarrays allowed for the simultaneous measurement of thousands of genes, enabling researchers to compare gene expression profiles between different placental conditions in a high-throughput manner without needing to formulate a hypothesis for a specific small set of genes (Tarca et al., 2006). However, microarrays were limited by their reliance on probe design based on known sequences, which restricted the discovery of novel transcripts.

In recent years, RNA sequencing (RNA-Seq) has largely supplanted microarrays as the gold standard in transcriptomics due to its ability to provide a more comprehensive and accurate view of the transcriptome (Yong & Chan, 2020). The RNA-Seq workflow involves library preparation, which includes RNA fragmentation, reverse transcription to complementary DNA (cDNA), adapter ligation, and PCR (polymerase chain reaction) amplification (Head et al., 2014). Sequencing is typically performed on high-throughput platforms, with Illumina systems being the most widely used (Goodwin et al., 2016). The depth of sequencing is an important consideration, with deeper sequencing allowing for detecting low-abundance transcripts but at a higher per-sample cost (Sims et al., 2014).

1.4.3. Data processing and analysis

Raw sequencing data undergoes several processing steps, including quality assessment of raw reads, trimming of low-quality bases and adapter sequences, alignment to a reference genome or transcriptome, and quantification of gene/transcript expression (Conesa et al., 2016; Patro et al., 2017). Quality control at this stage includes assessing mapping rates, duplication rates, and gene body coverage (L. Wang et al., 2012).

The principal aim of many placental transcriptome studies is to identify differentially expressed genes (DEGs) between conditions (e.g., normal vs. Complicated pregnancies). Popular tools for this analysis include DESeq2 and edgeR (Love et al., 2014; M. D. Robinson et al., 2009). This step typically involves normalization of count data, statistical testing for differential expression, and correction for multiple testing (Cox et al., 2015).

The final stage involves interpreting the biological significance of the transcriptomic data. Common approaches include pathway analysis, co-expression network analysis, gene set enrichment analysis (GSEA) and comparison with other publicly available datasets (Cox et al., 2015; Langfelder & Horvath, 2008; Subramanian et al., 2005). These analyses help to contextualize the DEGs within biological processes and pathways relevant to placental function and dysfunction.

1.4.4. Validation and emerging trends

While RNA-Seq provides a comprehensive view of the transcriptome, key findings are often validated using orthogonal methods. Quantitative reverse transcription PCR (RT-qPCR) remains the gold standard for validating expression changes in selected genes (Bustin et al., 2009). For protein-coding genes, validation at the protein level (e.g., by Western blot or immunohistochemistry) can provide additional confidence in the biological relevance of the findings (Eaton et al., 2013).

Recent developments in placental transcriptomics include single-cell RNA-Seq (scRNA-Seq) and spatial transcriptomics. These techniques require additional steps in sample preparation and data analysis but offer a much more detailed image of cellular heterogeneity and the spatial organization of gene expression in the placenta (Rodriques et al., 2019; Vento-Tormo et al., 2018). For instance, Vento-Tormo et al. (2018) used scRNA-Seq to create a single-cell reconstruction of the maternal-fetal interface in early pregnancy, revealing new cell subtypes and complex cellular communication networks (Vento-Tormo et al., 2018).

1.5. Summary of literature review

The placenta is a unique mammalian organ that is a crucial interface between the mother and the developing fetus during pregnancy. It's distinct genome and properties mediate rapid implantation, trophoblast invasion, proliferation, and differentiation, and it plays a vital role in nutrition, metabolism, growth, and development of the fetus while adapting to the mother's gestational needs. However, placental complications can lead to severe pregnancy complications such as miscarriage, preeclampsia, gestational diabetes, fetal growth restriction, and preterm birth. Recent advances in high-throughput RNA sequencing technology have made it possible to analyze the placental transcriptome in unprecedented detail, providing insights into the complex molecular mechanisms underlying placental development and functions. While adopting a new technology requires more debugging and is initially more expensive, it enables us to uncover the underlying biological processes in significantly increased detail. By understanding the placenta's crucial role in pregnancy and the molecular mechanisms underlying its development and function, researchers can develop new ways to diagnose, manage and potentially treat placental complications and improve maternal and fetal health outcomes.

2. AIMS OF THE STUDY

This thesis aimed to develop the experimental and analytical pipeline to generate and profile placental whole transcriptome sequencing datasets in normal and complicated pregnancies.

The specific objectives of the thesis are:

1. to assess the effect of the RNA degradation process in the placenta after the delivery on the RNA-Seq experiment results;
2. to characterize the landscape of differentially expressed genes (DEG) and involved biological pathways in early and late gestational complications;
3. to test the deviation of parental allelic expression of 91 imprinting candidate genes in the placenta and to analyze their transcript dynamics in the context of gestational status.

3. MATERIALS AND METHODS

3.1. Ethics

The protocols of REPROMETA (full study name: REPROgrammed fetal and/or maternal METAbolism) study were reviewed and accepted by the Ethics Review Committee of Human Research of the University of Tartu, Estonia (permissions no 146/18, 27.02.2006; 150/33, 18.06.2006; 158/80, 26.03.2007; 221/T-6, 7.12.2012; 286/M-18, 15.10.2018). Placental samples representing therapeutic medically induced abortion during the second trimester due to maternal medical risks of pregnancy and recurrent pregnancy loss (RPL) cases were analyzed upon ethics approvals no 117/9 (16.06.03) and 212/M-32 (20.02.2012).

All subjects provided written informed consent at the recruitment. All procedures and methods comply with the Declaration of Helsinki guidelines.

3.2. Study subjects

3.2.1. Recruitment

REPROMETA study participants were recruited, and the sample set was collected at the Women's Clinic of Tartu University Hospital in Estonia. The REPROMETA study focused on gathering clinical data and biological samples from singleton pregnancies occurring between 36 and 42 gestational weeks (g.w.). The study participants, consisting of family-placenta trios/duos, were recruited during 2006–2011 at the time of delivery. Cases involving fetal anomalies, chromosomal abnormalities, and inherited diseases, as well as pre-existing conditions like chronic hypertension, diabetes mellitus and chronic renal disease, were excluded from the study. All participants had a white European ancestry. Based on the newborn's birth weight and the presence or absence of maternal pregnancy-specific complications, the pregnancies of the REPROMETA study were classified into the following clinical subgroups – normal term pregnancy (Term norm; n=108), gestational diabetes (GD, n = 55), preeclampsia (PE, 61), delivery of a large for gestational age newborn (LGA, n = 113) or a small for gestational age newborn (SGA, n = 86). In total, 373 pregnancies were recruited, including 99 cases with an overlap between two or more clinical conditions (for example, PE + SGA).

In addition to the term placental samples, this study also included placental samples representing the first and second-trimester euploid pregnancies that had been terminated for various reasons and had been collected over an extended period spanning from 2003 to 2012. These samples were further categorized into three groups: electively terminated 1st trimester pregnancy (1st TRIM; n = 48), 2nd trimester pregnancy terminated due to a maternal health condition (2nd TRIM; n = 9), and samples from recurrent pregnancy loss (RPL; n = 2) cases. The same exclusion criteria employed in the main REPROMETA study were applied to

these additional samples. All of the samples originated from white European families, and placental karyotyping was performed to eliminate any gross chromosomal abnormalities in the analyzed placentas.

3.2.2. Clinical definitions

The original publications used for the doctoral theses analyzed RNA-Seq data from eight clinical groups (Table 2). Five of those groups were from term pregnancies (260–291 gestational days (g.d.)), one from the second trimester and two from the first trimester. The following two chapters describe the group characteristics in detail.

3.2.2.1. Term pregnancies (Studies I–II, IV)

The REPROMETA sample set included a control group of uncomplicated pregnancies with newborns of average birth weight for their gestational age (Term norm). The Term norm group consisted of uncomplicated pregnancies that resulted in the birth of a newborn with a weight appropriate for gestational age, meaning the birth weight fell between the 10^{th} and 90^{th} percentiles. The weight percentiles were calculated from the Estonian Medical Birth Registry (Karro et al., 1997). There were also study groups for small-for-gestational age (SGA) and large-for-gestational age (LGA) newborns. SGA and LGA groups were smaller than the 10^{th} percentile and larger than the 90^{th} percentile, respectively. The size variation groups used in this study were otherwise normal; any cross-classification with other complications was avoided.

Maternal pregnancy complications were represented by severe late-onset preeclampsia (LO-PE) and gestational diabetes mellitus (GD). PE cases were characterized by hypertension (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg) and/or proteinuria (≥5 g in 24 hours), with the first hypertension or proteinuria symptoms developing after 34th gestational week (as defined by the ACOG 2013). Four of the eight cases were also classified with intrauterine growth restriction (IUGR) after their last ultrasound examination demonstrated abnormal Doppler waveforms in the umbilical or middle cerebral artery and/or if the newborn's weight and/or abdominal circumference was $<10^{th}$ percentile while head circumference was $>10^{th}$ percentile. Gestational diabetes mellitus was diagnosed through oral glucose tolerance tests (75 g), conducted between 24 and 28 weeks of gestation, with fasting venous plasma glucose levels exceeding 4.8 mmol/l and/or plasma glucose levels at one hour and two hours after the test, respectively exceeded 10 mmol/l and 8.7 mmol/l (Panel, 2010).

3.2.2.2. First and second-trimester pregnancies (Studies III–IV)

First and second-trimester termination cases samples were divided into three groups – 1st TRIM, 2nd TRIM and RPL (recurrent pregnancy loss).

1st TRIM was comprised of normal first-trimester pregnancies with no maternal or fetal clinical complications until the termination of the pregnancy (51–81 g.d.). None of the patients had experienced any clinically confirmed pregnancy losses in their reproductive history.

2nd TRIM was comprised of medically induced abortions from the second trimester (126–167 g. days). In all of those cases, the medically induced termination of the pregnancy was due to maternal health indications without any known fetal complications. Fetal anomalies were excluded by the pathology specialist assessment.

RPL group comprised of two cases, both of whom had a series of clinically confirmed miscarriages before the index cases (respectively five and six). Both pregnancy losses occurred in the first trimester (44 and 67 g.d.) and at least a week before, normal growth and heartbeats were detected in ultrasound scans. Other known pregnancy loss risk factors, such as abnormal karyotype of partners, abnormal menstrual cycle, antiphospholipid syndrome, genital infections or thrombophilic mutations in female partners, were excluded before recruitment.

3.2.3. Selection of study samples

For the RNA-Seq experiment, we sequenced eight samples per group, except in the case of 2nd trim (n = 6) and RPL (n = 2) samples, for a total of 56 samples (Table 2). When choosing the samples, we aimed to include samples with the most distinct characteristics of the respective clinical groups. We also strived to balance gestational age and fetal sex. In the case of PE samples, half also had a compounding IUGR diagnosis. The final characteristics of the study groups are described below in Table 2.

In reference IV, genome-wide genotyping data from the placenta and parental blood samples were required to assess the parental origin of gene expression. Parental blood samples were not available for all pregnancies (n = 54), so the study analyzed 16 maternal-placental duos and 38 mother-father-placental trios.

Table 2: Clinical characteristics of the sampleset.

	1st trim	2nd trim	Term norm	PE	GD	LGA	SGA	RPL
STUDY	III, IV	III, IV	I–IV	II–IV	I, II, IV	I, II, IV	I, II, IV	III
n	8	6	8	8	8	8	8	2
Pre-pregnancy BMI (kg/m2)	22.2 (20–30)	19.7 (17–25.2)	23.8 (17–30)	26 (20–34)	25.5 (18–43)	24.2 (19–31)	21.1 (17–24)	20.3 (20– 21)
Gestational weight gain (kg)	NA	NA	18.8 (11–20)	10 (6–16)	13 (8–25)	20 (14–33)	12.8 (9–20)	NA
Nulliparity (n)	2	4	3	6	3	2	7	1
Smokers during pregnancy (n)	8	1	2	2	1	0	2	0
Gestational age at birth (weeks)	8.6 (7.3–11.6)	19.1 (18–21)	40.6 (37.1–41.6)	38 (37.1–38.7)	39.4 (38.3–40.4)	40.1 (39.3–41.1)	38.4 (37.7–41.3)	7.9 (6.3 – 9.6)
Labour activity (yes/No)	0/8	0/6	5/3	2/6	3/5	3/5	7/1	0/2
Delivery mode (vaginal/c-sect)	0/8	0/6	5/3	2/6	3/5	3/5	6/2	0/2
Newborn weight (g)	NA	330 (330–330)	3756 (3102–4220)	2803 (2170–3570)	4284 (3940–4680)	4744 (4420–4986)	2517 (2004–2698)	NA
Newborn length (cm)	NA	NA	51.2 (48.5–54.5)	48 (45–49)	52.5 (51–54)	53.2 (52–55)	46 (45–48)	NA
IUGR (n)	0	0	0	4	0	0	6	0
Newborn sex (F/M)	4/4	3/3	3/5	4/4	5/3	4/4	5/3	1/1
Placental weight (g)	NA	NA	575 (420–770)	462.5 (340–720)	587.5 (500–1060)	817.5 (610–970)	420 (200–470)	NA
RNA integrity (RIN)	7.9 (6.2–8.9)	7 (6.1–8.7)	6.8 (6.3–8.2)	6.7 (5.9–8)	6.8 (6.1–7.3)	7.2 (6.2–8.2)	6.6 (6.3–7.7)	7.9 (7.8– 7.9)

Units are marked in the first column, if the unit is not a count or fraction then the value follows a pattern median (minimum-maximum); *Ponderal index, a ratio of body weight to length: [weight (in g) x 100] ÷ [length (in cm)]; IUGR – intrauterine growth restriction; BMI, body mass index; RIN, RNA integrity number.

3.3. Study design

As mentioned in Chapter 2, the thesis sought to accomplish three main objectives: assess the effect of RNA degradation on RNA-Seq experiments, characterize differential gene expression in early and late pregnancy complications, and test parental allelic expression deviation of imprinting candidate genes in the placenta. The design of the thesis is elaborated below (Figure 2).

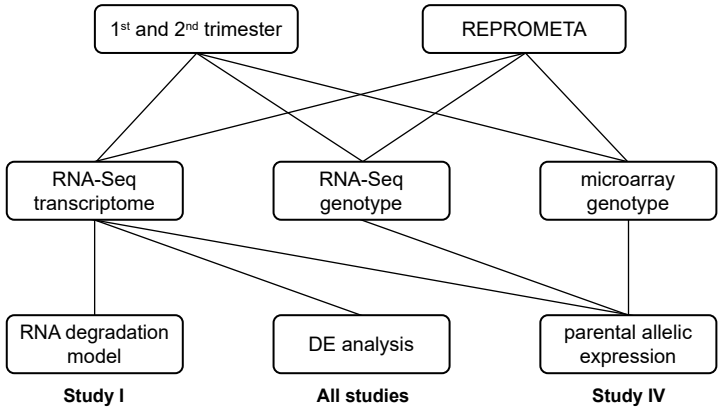

Figure 2: Thesis design. The thesis utilized two cohorts (row 1) across three different datasets (row 2) and conducted three distinct analyses to address the study's objectives (row 3). All of the term samples originated from the REPROMETA (REPROgrammed fetal and/or maternal METAbolism) study sample set, but the first and second trimester samples were recruited separately. In total, transcriptome and genotype analysis was performed on 56 placenta samples using RNA-seq, and most (n = 54, Table 2) also had their parental and placental blood samples genotyped with Illumina HumanOmniExpress-12-v1/24-v1 BeadChips. The datasets were then subjected to three main analytical approaches to address the three objectives of this thesis.

3.4. Placental RNA extraction and RNA sequencing (Study I–IV)

3.4.1. Placental and blood sampling

Placentas were kept at +4 °C after vaginal delivery or cesarean section and were sampled within an hour. Blocks of 2–3 cm from the middle region of the placenta were taken, and the collected tissue samples were washed with 1 × PBS to remove maternal blood contamination. Then, they were immediately placed into RNA later solution (Ambion Inc, Life Technologies) and stored at −80 °C until RNA isolation. The same medical personnel used the same protocol for all second and third-trimester samples.

First-trimester tissue samples were obtained immediately after surgical termination of pregnancy (1st TRIM group, n = 8) or surgical removal of the conceptus

(RPL group; n = 2) under general anaesthesia. The maternal tissue was removed by Piret Illison (United Laboratories, Tartu University Hospital) under a stereomicroscope (Discovery V8, Zeiss) and chorionic villi containing both cyto- and syncytiotrophoblast cells were placed into RNAlater solution (Ambion Inc, Life Technologies) and stored at −80 °C without any further manipulation. For all first-trimester samples, part of the purified trophoblast cell population was karyotyped and it confirmed typical male or female karyotype in all cases (United Laboratories, Tartu University Hospital).

Parental blood samples for term pregnancies (>37 g.w.) were collected at the delivery room. in Women's Clinic of Tartu University Hospital. Only maternal blood was collected in pregnancies terminated during 1st and 2nd trimester.

3.4.2. Total RNA extraction and quality control (QC)

Total RNA extraction of the placental samples utilized in this study was performed by three researchers (Mario Reiman; Jaana Männik, PhD; Kristiina Rull, MD, PhD) using the same protocol and equipment. Total RNA purification and quality control steps were all carried out by Mario Reiman.

The placental tissue sample was thawed on ice, and a 200- to 300-mg slice was cut from the middle of the sample and weighed. The tissue was homogenized with an IKA Ultra-Turrax T8 homogenizer (IKA Works, Staufen im Breisgau, Germany) in 4 ml Trizol reagent (15596018; Thermo Fisher Scientific). After homogenization, samples were stored in a –20 °C freezer until a set of 4–10 samples were gathered, and then the samples were thawed to room temperature, 800 µl of chloroform was added and tubes were vigorously shaken for about 2–3 minutes. The fully homogenized mixture was then centrifuged for 15 minutes (12'000 g, +4 °C) and the then formed top aqueous phase was pipetted to a new tube. Isopropanol was added in an equal amount of the obtained aqueous phase; the tube was vortexed for 30–60 seconds and then incubated for 10 minutes at room temperature (occasionally re-vortexing for a few seconds). The tube was then centrifuged for 20 minutes (16'000 g, room temperature) and as a result, the RNA formed a small pellet at the bottom of the tube. The supernatant was removed and the pellet was washed with 75% ethanol (+4 °C), after which the tube was once again centrifuged (7500g, +4 °C, 5 minutes) and the supernatant removed. The tube and pellet were then left to dry until the white RNA started turning transparent, after which the RNA was dissolved in RNase-free water. Dissolution in water was done by snipping and vortexing the tube until no pellet was visible and after that, the tube was heated at 57 °C for 10 minutes. After dissolution, 3µl of the sample was aliquoted for QC and the rest was stored in a –80 °C freezer. The purity level and concentration of isolated total RNA were measured using a NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific). RNA integrity was assessed with 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and was expressed as RIN (RNA integrity number) values. RIN has been commonly used as a measure of the integrity of

integrity of mRNA. The RIN ranges from 1 to 10, indicating low or high RNA integrity, respectively (Sonntag & Woo, 2018).

After all the samples had their RNA extracted and the QC results measured, all of the samples were further purified with RNeasy MinElute columns (74204; Qiagen, Germantown, MD, USA), according to the manufacturer's protocol. This was done because some of the RNA-s were showing potential contaminants in the spectrophotometer readings and to avoid potential batch effects, all of the samples were purified with the same process. After purification, further nanodrop measurement confirmed the high degree of purity in all samples.

3.4.3. RNA-Seq library preparation and sequencing

RNA-seq libraries were prepared at the Sequencing Core Laboratory of the Finnish Institute of Molecular Medicine (FIMM) by Mario Reiman and Siim Sõber, PhD under the guidance of Pirkko Mattila, PhD.

High-purity total RNA (5 μg) was ribodepleted with Ribo-Zero rRNA Removal Kit (MRZH11124; Illumina, San Diego, CA, USA) and then purified (NucleoSpin® RNA Clean-up XS, Macherey-Nagel, Duren, Germany). Reverse transcription to double-stranded DNA was done with SuperScrip Double-Stranded cDNA Synthesis Kit (Life Technologies, Carlsbad, CA, USA), with random hexamers (New England BioLabs, Ipswich, MA, USA) used for priming the first strand synthesis reaction and SPRI beads (Agencourt AMPure XP, Beckman Coulter, Brea, CA, USA) for purification of cDNA.

Nextera Technology (Illumina, San Diego, CA, USA) was used to prepare RNA-Seq Libraries. DNA fragmentation and tagging were performed by in vitro cut-and-paste transposition. To add the Illumina-specific bridgePCR compatible sites and bar codes and enrich the library, limited-cycle PCR (5 cycles) was done according to the instructions of the Nextera system. SPRI beads were used to purify the PCR products, and the library QC was evaluated by Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

The sequencing was performed by FIMM Sequencing Core Laboratory personnel. C-Bot (TruSeq PE Cluster Kit v3, Illumina, San Diego, CA, USA) was used for cluster generation and Illumina HiSeq2000 platform (HiSeq TruSeq v3 reagent kit) for paired-end sequencing with 46 bp read length. In the case of the first trial run (n = 2), we used 101 bp read lengths. Each transcriptome was loaded to occupy 1/3 of the lane capacity in a flow cell.

3.4.4. Placental and parental genotyping (study IV)

Placental or chorionic villus samples were collected and stored in dry cryovials for DNA genotyping. For RNA studies, the samples were placed in RNAlater solution and kept at −80 °C until DNA isolation. The genotyping of placental and blood genomic DNA was carried out using Illumina HumanOmniExpress-12-v1/24-v1 BeadChips, which had a median spacing of 2.1 kb and over 715,000 markers.

3.5. Data analysis

3.5.1. Base bioinformatics of the placental RNA-Seq dataset

The base bioinformatics of the generated RNA-Seq dataset was conducted using the RNA-Seq pipeline v.2.4 (Sequencing Core Laboratory; FIMM, Helsinki, Finland). Reads were filtered for quality, adapter, rRNA and mitochondrial DNA sequences, and homopolymer stretches using custom Python (Van Rossum, 1995) scripts. Read alignment to human genome assembly (GRCh37.p7/hg19) was done with TopHat v.2.0.3 (Kim et al., 2013). Transcript quantification, measured as fragments per kilobase per million (FPKM), was conducted with Cufflinks v.2.0.2 (Trapnell et al., 2010) with reference annotation (gene annotations from Ensembl v.67) (Kersey et al., 2016) and gene expression was quantified by HTSeq analysis (Anders et al., 2015).

3.5.2. Placental RNA degradation modelling (Study I)

Before modelling and testing for a correlation between RIN and RNA-Seq-detected gene expression, a set of other metrics was tested for correlation with RIN to include/exclude them from the final model. Student's t-test was used to test clinical group (Term norm, GD, LGA and SGA), newborn gender (male vs. female) and delivery mode (vaginal or Caesarian section). Pearson correlation between RIN and the following metrics were tested for: gestational age; the proportion of reads that passed the read filtering phase of RNA-Seq pipeline; efficiency of alignment among the filtered reads, the ratio of coding bases, proportion of ribosomal bases, proportion of UTR bases, proportion of intronic bases, the proportion of intergenic reads, median 5' bias of 1000 most highly expressed transcripts and median 3' bias of 1000 most highly expressed transcripts. Since no metric showed any statistically significant correlation to RIN, no covariates were included in the RNA degradation model.

Transcript abundance was modelled in DESeq2 using built-in read count normalization, RIN was set as a continuous explanatory variable, and gene expression as a dependent variable. Raw read counts from the htseq-count for Term norm, GD, SGA, and LGA samples (n = 32) were used as input. Of the initial 53,893 Ensembl v.67 genes with available read counts, 39,220 genes were excluded as transcriptional noise (mean fragment count <50) and a further 135 were excluded by outlier detection by Cook's cutoff (95^{th} percentile of F distribution), leaving 14,538 genes for statistical testing. Differential expression (DE) relative to the RIN values was tested using a likelihood ratio test to compare nested models. Raw read counts from the htseq-count for Term norm, GD, SGA, and LGA samples (n = 32) were used as input. The error model followed a negative binomial distribution and a logarithmic link was used on counts (default methods in DESeq2).

Since the results showed a small but pervasive RIN effect on all genes, we did not define differentially expressed genes with a p-value cutoff, and instead, three

gene groups were established. These groups were used when we wanted to compare the differences between the slowly and rapidly degrading genes. The first group, labelled 'Negative', consisted of genes (n = 727) displaying a negative correlation with RIN, indicating the lowest $\log_2$ fold change values (5% of genes). The second group, termed 'None', encompassed genes (n = 727) with no correlation to RIN, as their $\log_2$ fold change values were closest to 0 (5% of genes). Lastly, the third group, designated 'Positive', comprised genes (n = 727) exhibiting a positive correlation with RIN, representing the highest $\log_2$ fold change values (5% of genes). The categorization of genes into these groups was validated by performing the differential expression tests and regrouping the genes in 1000 random subsamples (n = 16) of the whole sample set. Successive statistical analysis was conducted to compare the characteristics of the transcripts encoded by genes in the 'Negative' group to those in the 'Positive' group.

3.5.3. Placental differential gene expression analysis (Study II, III)

Differential expression in RNA-Seq data was tested using DESeq (Anders & Huber, 2010) and DESeq2 (Love et al., 2014) packages for R (R Development Core Team, 2018). Read counts from htseq-count were used as input and normalization was done with built-in algorithms of DESeq and DESeq2. Outlier detection and handling were performed using the default method in DESeq. In DESeq2, outliers were replaced using the *Replace outliers with trimmed mean* function with default Cook's distance cutoff. Statistical testing indicated that the two software packages, DESeq and DESeq2, differ substantially in their sensitivity and robustness in assessing differential expression. Compared to the seminal DESeq package, analysis with the more recently developed DESeq2 program produced a markedly higher number of significant results for all conducted differential expression tests with our data. Thus, in studies II and III, a more stringent level of significance was imposed on the test results of DESeq2. A gene was considered differentially expressed when the statistical tests simultaneously satisfied the following empirically set thresholds: false discovery rate (FDR) < 0.1 for DESeq and FDR <0.05 for DESeq2. Only genes with mean normalized expression greater than 50 fragment counts were included in the analysis to exclude noise from lowly expressed loci. No covariates were automatically included in the tested models. Instead, potential confounders (delivery mode, initiated labour activity, gestational age, gender, placental weight, birth weight/height, maternal pre-pregnancy BMI, weight gain, age and parity) were tested independently for the differential expression effect on all genes included in the analysis. For quantitative variables, the samples were divided at the median value of the parameter.

3.5.4. Analysis of parental transcription ratios (Study IV)

A list of candidate genes for parent-of-origin determined allelic expression was retrieved from the Geneimprint database (n = 300, imprinting candidate gene list as of 2014) (Geneimprint : Genes, last accessed 2018) and articles that reported novel candidate imprinted genes in the human placenta (n = 96) (Court et al., 2014; Hamada et al., 2016; Metsalu et al., 2014). Total number of genes entering the analysis pipeline was 396.

To confidently determine the parental origin of placental transcripts, a strict filtering pipeline and data quality control were applied, developed by Diana Pilvar. First, genes not annotated in Ensembl v.67 and genes with too low (<50 fragments per sample) median expression were filtered out. For the remaining 207 genes, the Ensembl Biomart tool (Kinsella et al., 2011) was used to identify common biallelic exonic SNPs (MAF > 10%) in the available parental-placental genotyping dataset. In study IV, SNP-s with genotype distributions deviating from Hardy-Weinberg equilibrium (P > 0.05) and SNPs with no or with too few (<3 trios/duos) informative families were excluded.

Next, maternal and paternal read counts at selected marker SNP positions for each gene were determined from the placental RNA-Seq dataset of the informative families (BAM files) with samtools mpileup command (parameters: –ABQ 0, reference genome GRCh37.p7) (Li, 2011). SNPs located within alternative exons overlapping with introns of the main transcript and SNPs with < 3 median reads at the variant position across informative placentas were excluded after manual inspection of RNA-seq reads using IGV 3.0 software (performed by Diana Pilvar) (J. T. Robinson et al., 2011).

For the remaining 91 genes, the proportions of maternal (Mat) and paternal (Pat) reads across all samples were calculated and the outcome was expressed as the Mat/Pat reads ratio along with the estimated 95% confidence interval (CI). The observed parental transcript ratios were statistically tested assuming equal expression levels for both alleles, using a binomial test in R. A gene was considered imprinted if at least 90% of the RNA-Seq reads were assigned to one parental allele, indicating close to monoallelic expression in a parent-of-origin-specific manner. Genes showing a statistically significant deviation from the expected maternal/paternal transcript ratio, with ≥ 65% but < 90% reads originating from one parental allele, were classified as exhibiting biased parental allelic expression. Genes were categorized as biallelic when the proportions of parental reads did not significantly differ from the expected ratio (Bonferroni corrected $P_{corr} > 0.05$) and/or the estimated proportions of both parental allelic reads fell within 35–65%.

3.5.5. Other implemented data analysis and statistical testing methods (Study I–IV)

Gene set enrichment analysis was performed using g:Profiler (Reimand et al., 2007). In study I, enrichment was tested for categories related to Gene Ontology; studies II and III added KEGG pathways and transcription factor (TF) regulatory motifs. For a later comparison of studies II and III, I used all databases available for the program. Studies II and III used genes that were deemed differentially expressed as input gene sets; in study I, a list of the top 5% of the most rapidly and slowly degrading genes was used instead of a list of DE genes.

Statistical testing, principal component analysis (PCA) and hierarchical clustering and plotting were performed in R. Plot annotations were finalized in Adobe Illustrator (Adobe Illustrator).

3.5.6. My contributions to the studies of this thesis

My role and responsibilities in those studies varied from study to study (Table 3). However, I was responsible for the primary wet-lab experiments to generate the data utilized in all four publications of the PhD thesis – placental RNA extraction and purification, and RNA-Seq library synthesis.

Table 3: My contributions to original research articles included in the thesis.

Steps and methods of the studies	Reiman 2017	Sõber 2015	Sõber 2016	Pilvar and Reiman 2019
Study design	Major	no	no	no
RNA extraction	Major	Major	Major	Major
Library prep and RNA-Seq	Major	Major	Major	Major
DNA genotyping	n.a.	n.a.	n.a.	no
QC/filtering	Major	no	no	Minor
Data analysis	Major	no	no	Major
Validation	n.a.	no	no	no
Result interpetation	Major	no	no	Minor
Drafting the manuscript	Major	no	no	no
Manuscript revision	Major	Minor	Minor	Major

"n.a." study did not contain this step, "no" no practical involvement, "Minor" small contribution, "Major" significant contribution

4. RESULTS

4.1. Characteristics of the generated and analyzed placental RNA-Seq dataset

The combined RNA-Seq dataset of 56 placental samples generated during the four substudies of these doctoral theses was comprised of 2.39×10^9 paired-end read pairs. On average, each sample contained 42.6×10^6 read pairs, ranging from 27.3 to 74.6×10^6. However, after filtering and annotation, the resulting fragment count from HTSeq was reduced to 1.53×10^9. On average, each sample contained 27.3×10^6 fragment counts, ranging from 15.8 to 56.9×10^6. Additionally, the dataset was filtered by the mean expression per gene, specific to each experiment and the compared groups. For reference, Table 4 marks the number of genes per group with a mean of over 50 fragment counts per sample, which correlates well with the number of genes involved in all the differential expression analyses. It is not the exact number of tested genes because each pairwise test had the mean expression filter stage applied to it, but generally, the number of tested genes in each test was between the numbers of individual group gene numbers.

Table 4: Sample set library sizes. The first two columns show the mean, minimum and maximum in millions; the last column is the count of genes with a greater than 50 counts mean expression in the respective samples.

group	n	Sequenced fragments ($\times 10^6$)	HTSeq counts ($\times 10^6$)	Genes with >50 mean counts (n)
1st trim	8	42 (28.1–48.2)	28.5 (18.2–37.4)	14 404
2nd trim	6	41.9 (35.5–51.7)	25.3 (15.8–36)	15 436
Term norm	8	39.4 (28.2–49.1)	24.9 (18.6–31.7)	14 445
PE	8	44.7 (35.2–53.6)	27.1 (19.6–38)	14 663
GD	8	39.6 (27.3–48.3)	25.8 (17.7–35.1)	13 904
LGA	8	45.2 (31.9–74.6)	26.9 (18.6–35.7)	14 909
SGA	8	42.9 (30.1–52.1)	27.5 (19.9–33.7)	15 214
RPL	2	52.3 (41.3–63.3)	44.7 (32.4–56.9)	16 559

4.2. RNA integrity modulates RNA-Seq profile (Study I)

Study I aimed to assess the role and extent of RNA degradation in modulating the outcome of RNA-Seq-based gene expression profiling. The base assumption was that the transcript pool of all genes is modulated by RNA degradation to some extent, but some mRNAs are degraded with an increased speed and on the contrary, some are more stable. This biological process will be reflected in the negative

(slowly degrading) or positive correlation (rapidly degrading) between the sample RIN values measured before RNA-sequencing library preparation and estimated gene expression levels in the generated transcriptome dataset (Figure 3 and 4).

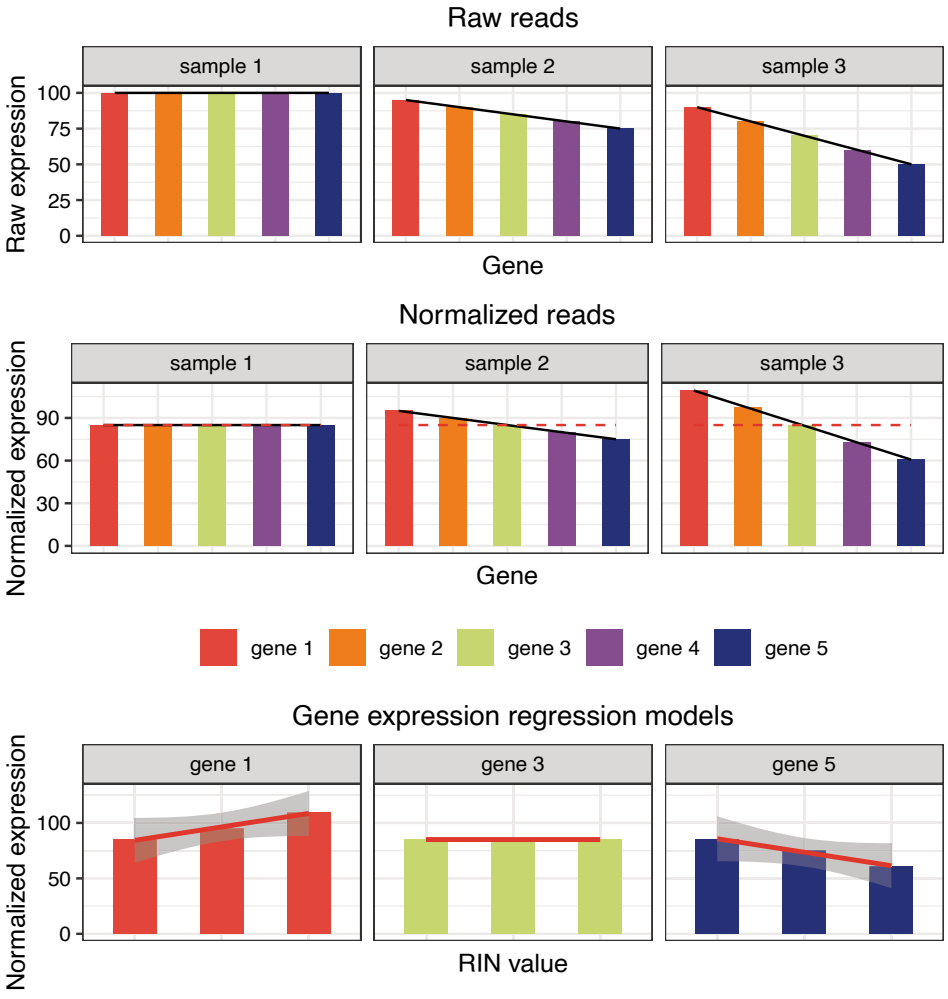

Figure 3: Schematic that describes how RNA degradation could cause differential expression to show up when, in reality, there was none. For example, we have three samples aliquoted from a single sample where sample 1 has had no RNA degradation, sample 2 has had some, and sample 3 had even greater degradation. After sequencing these samples, we could see raw read count expressions as shown in the first row; after normalizing for library size, the resulting image would be like shown in the second row. Since initially, all the samples came from the same sample, there should be no difference between the gene expressions, but we see that there would be a rather big difference in the case of gene 1 and gene 5 expressions and no difference in gene 3's case. That is because gene 1 is very resistant to degradation, gene 5 is very susceptible to degradation, and gene 3 degrades at an average rate.

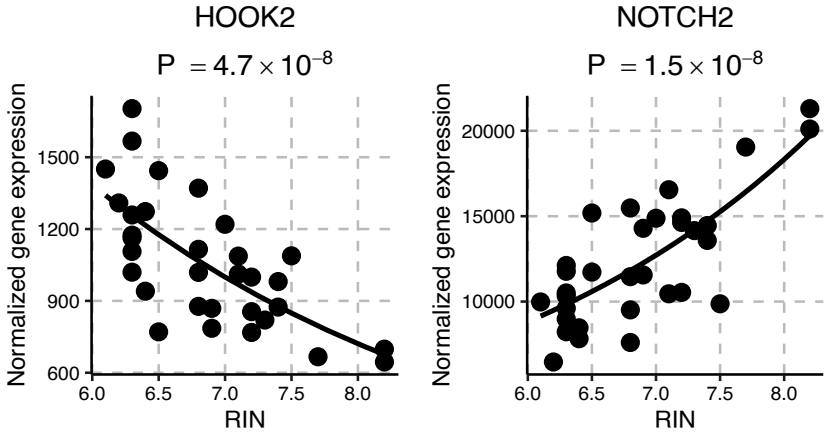

Figure 4: Top two genes with the lowest detected P values from the RIN-dependent differential expression analysis. Modified from Ref. I.

4.2.1. RNA integrity impacts transcript quantification in human placenta

In order to assess the impact of RIN-dependent perturbations on transcript level estimates, gene expression levels were modelled in DESeq2 with the explanatory variable as the sample RIN value. After removing low read count genes and models with outliers, 14,538 genes were tested. In samples with lower RIN values, RNA degradation is expected to affect mRNAs differently depending on their properties. This leads to the artificial enrichment of relatively stable transcripts and the depletion of quickly degraded transcripts (Figure 3). In total, 1,177 (8.1%) genes were detected with statistically significant RIN-dependent alterations in expression levels (false discovery rate (FDR) <0.1). Of those roughly half, 639 genes negatively correlated to RIN (indicating slower than average degradation rate) and 538 genes displayed positive correlations (faster than average degradation rate). The two genes with the most significant correlation between RIN values and gene expression levels are *HOOK2*, with a negative correlation to RIN indicating a slow mRNA degradation, and *NOTCH2*, with a positive correlation referring to accelerated RNA degradation (Figure 4).

The observed P values displayed a systematic skew towards smaller-than-expected P values (Figure 5 A). This shows that the RIN-gene expression correlation affects not only a small subsection of genes but almost all of the genes, including the ones that might not pass our FDR criteria of 0.1.

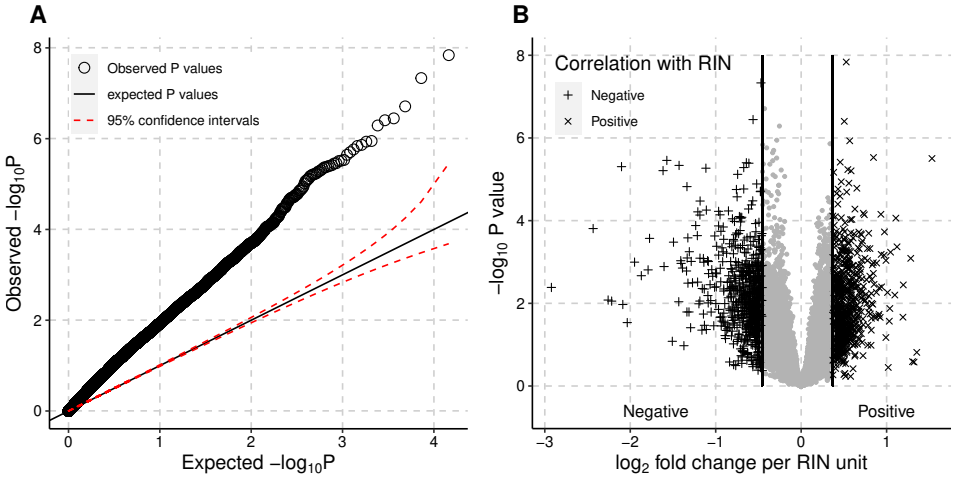

Figure 5: Results of testing for RIN-dependent differential expression in placental RNA-Seq samples. A) Quantile–quantile plot of *P* values from modelling a RIN-dependent effect on gene expression. The expected P values line shows where the P values would have placed if no statistically significant correlation between RIN and gene expression was detected. B) Volcano plot of the same P values and respective $\log_2$ fold change values. The subgroups formed from the 5% of the transcripts in both extreme ends of the distribution were used in subsequent analyses (each group; n = 727). Modified from Ref. I.

4.2.2. Gene groups with different degradation speeds have distinct biological functions and transcript features

To investigate whether genes with a slow or fast rate of degradation exhibit different biological and/or physical properties, two gene groups, referred to as 'Negative' and 'Positive,' were formed for further inspection. Both groups comprised 5% of the most extreme genes regarding the $\log_2$ fold change between RIN and gene expression (Figure 5 B).

The gene set enrichment analysis for gene ontology (GO) terms was performed using g:Profiler for the 'Negative', 'None', and 'Positive' gene groups. The initial number of genes in each group was 727. However, due to a lack of GO annotations associated with some genes, the final 'Negative' gene group included 630 genes and the 'Positive' gene group comprised 439 genes. The analysis identified 20 significantly enriched GO terms for genes with slower degradation rates. In contrast, genes with faster degradation rates showed only four significantly enriched GO terms (Table 5).

Table 5. Results of gene set enrichment analysis of gene ontologies (GO) terms for gene groups with positive and negative correlations between expression level estimates and RIN value using g:Profiler.

Name	% of pathway	% of query	P value[a]
Genes with negative correlation to RIN (N = 630)			
CC: extracellular region part	6	34.3	9.52×10^{-26}
BP: regulation of response to stimulus	5.9	9.2	3.13×10^{-17}
CC: collagen trimer	7.3	7	4.29×10^{-8}
CC: MHC protein complex	7.9	4.8	3.81×10^{-7}
CC: plasma membrane part	10.4	3.2	3.25×10^{-6}
CC: endoplasmic reticulum lumen	40	1	3.11×10^{-5}
MF: protein complex binding	5.8	9.8	1.12×10^{-4}
BP: reactive oxygen species metabolic process	13	2.2	2.58×10^{-4}
MF: glycosaminoglycan binding	10.9	4.1	4.29×10^{-4}
BP: platelet degranulation	16.5	2.2	1.92×10^{-3}
BP: endocytosis	5.5	21.4	2.38×10^{-3}
MF: antioxidant activity	20	1.4	5.68×10^{-3}
MF: extracellular matrix structural constituent	12.5	4	7.81×10^{-3}
BP: oxygen transport	44	1.7	1.40×10^{-2}
BP: regulation of muscle system process	21.7	3.2	1.64×10^{-2}
BP: negative regulation of molecular function	6.5	38.3	2.95×10^{-2}
BP: negative regulation of protein phosphorylation	17.6	1.9	3.08×10^{-2}
BP: chemical homeostasis	17.1	1.9	3.12×10^{-2}
BP: response to vitamin	11.7	3.7	3.53×10^{-2}
CC: clathrin-coated endocytic vesicle membrane	6.8	10	3.81×10^{-2}
Genes with positive correlation to RIN (N = 439)			
CC: intrinsic component of membrane	4	13.9	5.20×10^{-8}
MF: cation transmembrane transporter activity	3.4	44.9	7.31×10^{-3}
MF: secondary active transmembrane transporter activity	5.5	7.7	1.28×10^{-2}
BP: ion transport	8.1	4.1	2.93×10^{-2}

[a] – P value correction by g:SCS method; BP – biological process; CC – cellular component; MF – molecular function

I also analysed the distribution of transcript biotypes between gene groups and found that the frequency of transcripts with different biotypes varied significantly between degradation speed groups. Transcripts of pseudogenes and non-coding genes tended to have slower degradation rates, while transcripts with faster degradation rates were predominantly from protein-coding genes (refer to Table 6). I also observed that mRNAs and sncRNAs with a negative correlation to RIN

values had significantly shorter transcripts, 3'UTRs and a higher GC content than the genes in the positive group. Interestingly, the two genes with the lowest detected P values from the RIN-dependent differential expression analysis (*HOOK2* and *NOTCH2*, see Figure 4) aligned with the statistically significant effect directions from Table 6. *HOOK2*, with a negative correlation, exhibited a smaller transcript length (2604 bp vs. 11389 bp), shorter 3'UTR (278 bp vs. 3752 bp) and a higher GC% (62.0% vs. 49.4%) compared to *NOTCH2*, which had a positive correlation to RIN. These findings suggest that the physical properties and sequence composition of RNA molecules play a role in influencing transcript degradation rates.

Table 6: Transcript parameters in the gene groups exhibiting negative and positive correlation between RNA-Seq read counts and RIN values. Based on Ref. I.

Parameter	Category	Negative, n = 727	Positive, n = 727	Test[a]	P
Transcript biotype[b] [n (% of all transcripts)]	Protein coding	608 (83.6%)	429 (59.0%)	F	**1.5×10^{-25}**
	Pseudo-genes	24 (3.3%)	88 (13.1%)	F	**1.97×10^{-10}**
	lncRNA	69 (9.5%)	187 (25.7%)	F	**2.82×10^{-16}**
	sncRNA	37 (5.1%)	78 (10.7%)	F	**8.77×10^{-5}**
GC-base pair content (mean %)	Protein coding	52.8%	48.4%	W	**1.24×10^{-14}**
	Pseudo-genes	54.0%	47.0%	W	**1.13×10^{-3}**
	lncRNA	50.2%	47.1%	W	**6.41×10^{-5}**
	sncRNA	49.2%	48.8%	W	7.39×10^{-1}
Transcript length [bp, median (range)]	Protein coding	2389 (395–18,254)	3664 (507–18,883)	W	**$<2.2 \times 10^{-16}$**
	Pseudo-genes	1005 (374–5669)	843 (174–11,816)	W	1.69×10^{-1}
	lncRNA	2282 (300–12,167)	1579 (174–10,425)	W	**1.32×10^{-3}**
	sncRNA	164 (75–4,489)	392 (71–4,972)	W	**2.06×10^{-6}**
UTR length [bp, median (range)]	3' UTR	681 (0–7,660)	1587 (0–11,970)	W	**$<2.2 \times 10^{-16}$**
	5' UTR	177 (0–4,543)	215 (0–3,200)	W	7.60×10^{-3}

[a] F, Fisher's exact test comparing Negative and Positive groups of transcripts; W, Wilcoxon exact test comparing Negative and Positive groups of transcripts.
[b] Overall distribution of categories among the 4 groups of transcript biotypes was tested with Fisher's exact test with 4×10^6 Monte Carlo simulation replicates, $P = 2.5 \times 10^{-7}$

4.2.3. Take home message from analyzing the correlation between gene expression and sample RIN number

Our results indicate that small differences in RNA integrity affect transcript expression quantification by introducing a moderate and pervasive bias in expression level estimates. There are statistically significant biological (e.g., protein-coding vs. non-coding) and physical differences (e.g., length, GC%) between the transcripts that were degrading rapidly and slowly.

4.3. Placental differential gene expression in pregnancy complications (Study II–III)

The objective of Studies II and III was to analyse the placental transcriptome and detect differentially expressed genes in several major pregnancy complications, such as maternal late-onset preeclampsia (LO-PE), gestational diabetes (GD) and pregnancies with the delivery of either small-for-gestational-age (SGA) or large-for-gestational-age (LGA) newborns (Study II), as well as early recurrent pregnancy loss (RPL) (Study III). In these studies, RIN values from Study I were not incorporated as a cofactor in differential expression testing models, as the clinical groups' average RIN values did not significantly differ from one another, and the sample size in the groups did not allow for many variables to be used in the models.

4.3.1. Preeclamptic placentas exhibit major transcriptional perturbations (Study II)

Differential gene expression was investigated in the placental transcriptome of pregnant women with LO-PE and GD, and in affected fetal growth conditions (SGA, LGA) compared to normal uncomplicated term pregnancies (NORM). Among the complications studied, preeclamptic placentae showed a significant shift in the expression profile of hundreds of genes, with 215 genes showing significant differential expression (Figure 6 A). It is worth noting that 80% of the differentially expressed genes in LO-PE placentas had significantly lower transcript levels compared to the NORM group, including well-known genes implicated in placental function and preeclampsia.

In contrast, only a few transcripts showed significant differential expression in other complications (Figure 6 A). The results of the Principal Component Analysis (PCA) showed a clear separation between placental samples from LO-PE and NORM groups (Figure 6 B). While the GD group had some overlap with the NORM group, the placental gene expression profile in cases of SGA and LGA was more scattered and partially overlapped with the PE and GD groups.

However, it is important to note that the delivery date in PE pregnancies was 12.5 days earlier, on average than in the normal gestation group (Table 2). As a result, the potential confounding effect of gestational age could not be entirely ruled out. The correlation of gestational age with the expression of any genes was

tested and only four genes were identified with a statistically significant result (*SASH1, LEP, NRIP1, AF127577.11*), all of which were also among the significant results for PE. However, since these were only four genes, one of which (LEP) is a known PE biomarker, it was concluded that the characterized differential expression profile primarily reflects the disturbed placental transcriptome in preeclamptic pregnancies.

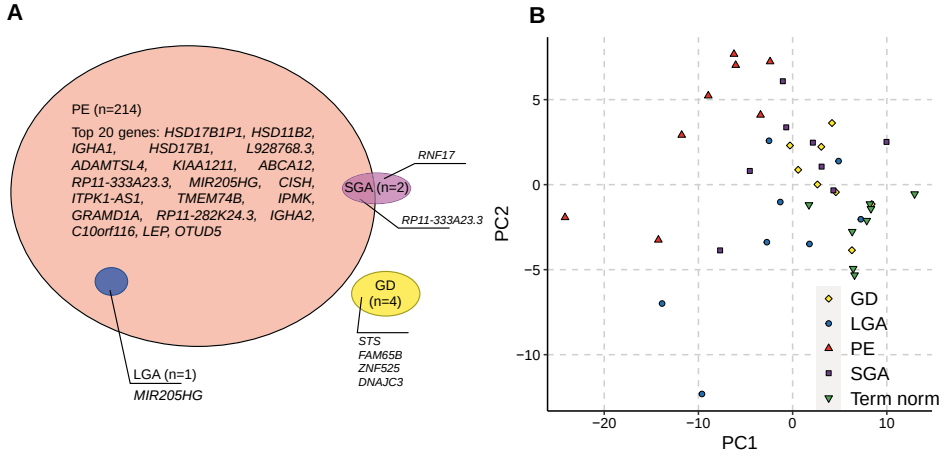

Figure 6: RNA-Seq analysis of 40 term placental samples (n = 8/group) reveals differential gene expression in cases of preeclampsia (PE), small- and large-for-gestational-age newborns (SGA, LGA) and gestational diabetes (GD) when compared to normal pregnancy (Normal). The analysis includes A) a Venn diagram illustrating differentially expressed genes in each pathology group and B) a Principal component analysis (PCA) showcasing the first two components. Modified from Ref. II.

4.3.2. Late pregnancy complications share signatures of placental malfunction

To explore the possibility of shared placental pathophysiology in pregnancy complications, we examined the estimated differential expression effects of the top 200 genes from RNA-Seq analyses of placentas affected by LO-PE, GD, SGA, and LGA, irrespective of statistical significance. The findings indicate that SGA and LO-PE placentas exhibited the highest concordance in gene expression disturbances compared to normal pregnancy, with a correlation coefficient of $R^2 = 0.70$ (linear regression, $P = 2.7\ 10^{-96}$; Figure 7 A). A strong correlation between the effects of top-200 genes in LO-PE and LGA placentas was also identified, with an R^2 value of 0.60 (Figure 7 B). Moreover, a correlation between the expression changes of the top-200 genes in SGA and LGA placentas compared to normal pregnancy was detected ($R^2 = 0.45$; Figure 7 C). Among these genes, 65 exhibited opposite effect directions, including well-known markers of aberrant placental development (*LEP, FLT, ENG, HTRA4, SH3PXD2A*). The gene expression profile of top-200 genes in GD placentas showed less pronounced overlap with other pathologies ($R^2 = 0.13–0.29$; Figure 7 D–F).

The correlation analysis showed that among the top 200 genes, the PE and LGA (n = 57/200 top genes) and the PE and SGA comparisons (n = 33) had the highest number of overlapping genes. This was supported by the hypergeometric test for enrichment ($P < 4.44 \times 10^{-16}$; Fig J). SGA and LGA shared 22 genes, of which 10 were shared only between the two. GD had lesser overlap with other conditions, but it was still statistically significant (PE, n = 14; LGA, SGA, n = 10 genes; $P < 4.71 \times 10^{-4}$).

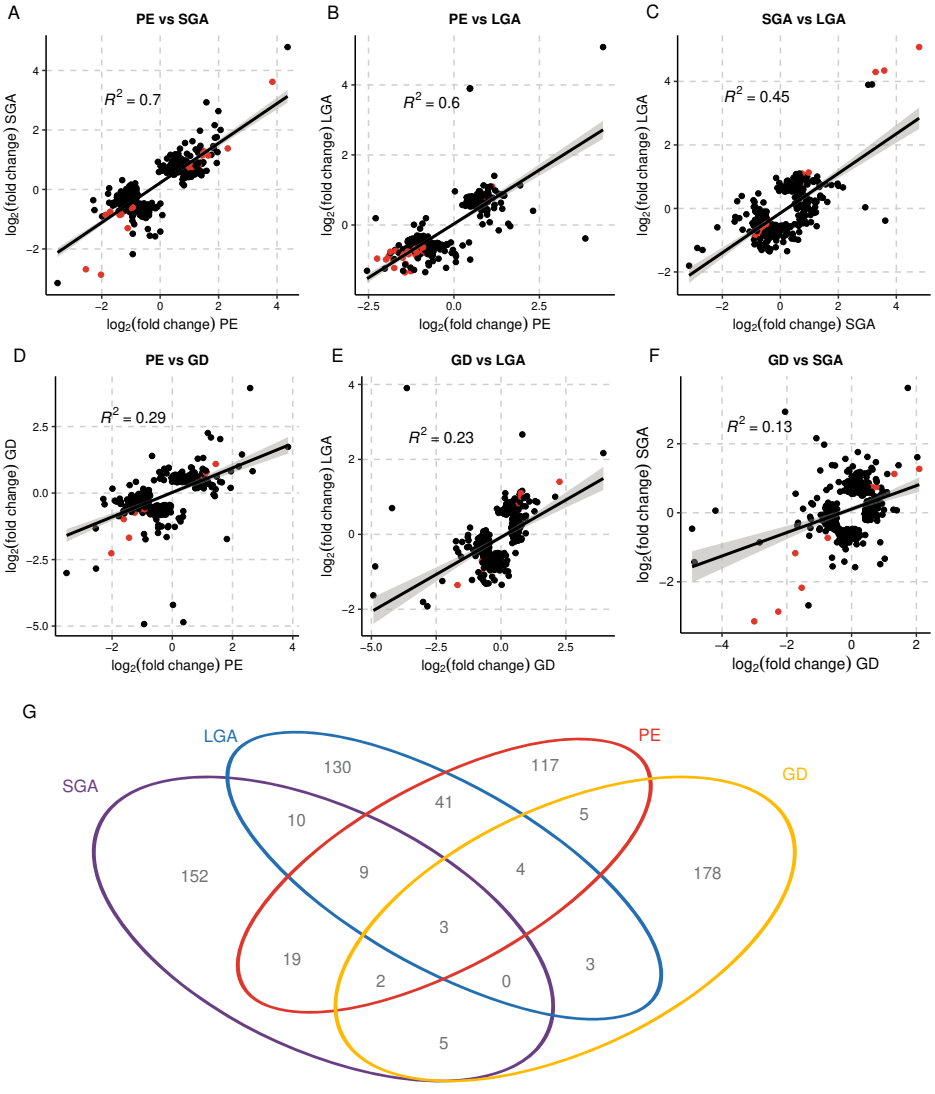

Figure 7: The graphs A-F show correlation plots of the 200 statistically most significant genes (by DEseq P values) from the DE analysis of each group. The red dots indicate the genes that are common between the gene lists. The R^2 value represents the correlation coefficient. Graph G displays a Venn diagram that illustrates the shared fraction of the top-ranked genes in the differential expression testing of different pregnancy complications. Modified from Ref. II

4.3.3. Chorionic villous transcriptome from recurrent pregnancy loss cases – malfunction of basic nuclear and cellular processes (Study III)

The transcriptome of first-trimester chorionic villous samples collected from two RPL cases was characterized and compared to gene expression in eight chorionic villous samples from uncomplicated first-trimester gestations (1st TRIM). The expression levels of a large number of genes showed a significant shift, with 189 genes meeting the significance criteria applied in the study. Of these genes, 27% (n = 51) showed significantly higher expression, and 73% (n = 138) showed significantly lower expression in RPL compared to 1st TRIM placentas. The functional categories of over- and under-expressed genes were distinct. Upregulated genes primarily involved protein processing/transport, fetal development, cell adhesion, immune response, and transcriptional regulation (Figure 8). In total, 13 of the 51 genes exhibiting increased expression in the RPL samples were supported by previous evidence for involvement in placental function and/or pathology (Figure 8).

In contrast, most genes exhibiting decreased expression were various snRNAs/snoRNAs, as well as ncRNAs involved in transcriptional, telomere, spliceosome, cellular protein traffic complexes, along with components responsible for the basic cellular apparatus for chromatin assembly and mitochondrial function (Figure 8 B). These processes are critical to the rapid proliferation and invasion of trophoblast in early pregnancy.

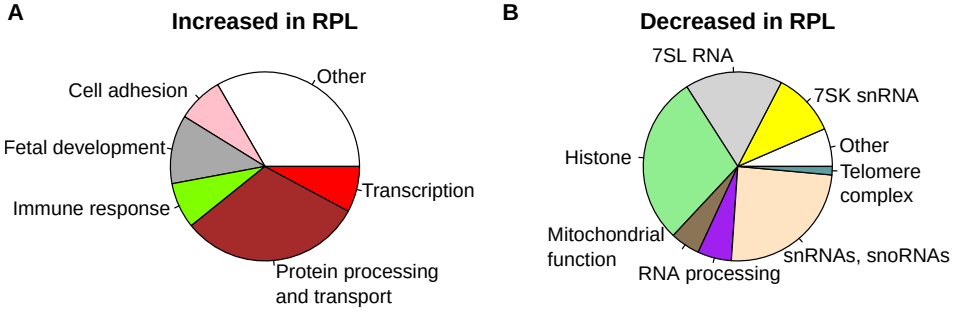

Figure 8: Pie charts of functional categories of genes with A) elevated (n = 51) and B) reduced (n = 138) expression. Modified from Ref. III.

4.3.4. Comparison of the transcriptome alterations in preeclampsia and recurrent pregnancy loss

Notably, differential expression of late-onset preeclampsia and first trimester recurrent pregnancy loss compared to gestational age-matched control samples showed a similar number of genes with altered transcript levels (215 and 189 genes, respectively). Therefore, I sought to investigate the degree of overlap between the two gene placental sets associated with abnormal pregnancy outocmes. A total of 399 distinct genes were identified across both datasets, with five genes

(*HIST1H4F, HIST2H2AC, HIST1H2AE, GRINA* and *PIGT*) found to be shared between the two studies (Figure 9, red dots). Interestingly, the direction of regulation for these five genes was the opposite in the two analyzed conditions. Specifically, the three histone-related genes were downregulated in RPL placentae and upregulated in PE, while *GRINA* and *PIGT* were upregulated in RPL and downregulated in PE. Upregulation of *GRINA* and *PIGT* gene expressions has been associated with increased metastasis and cell migration in various cancers (Islam et al., 2021; H. Ma et al., 2020; Tan et al., 2024).

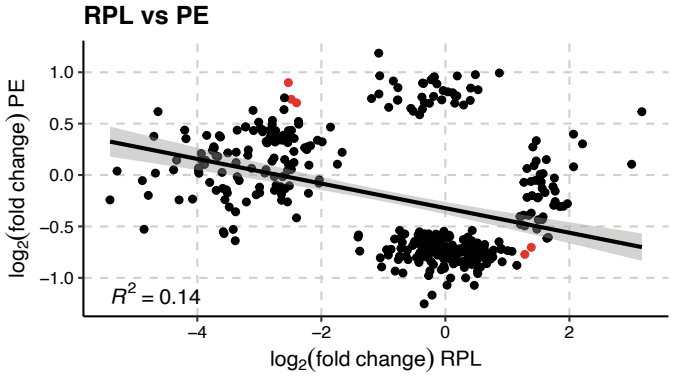

Figure 9: Dotplot of gene expression log2 fold changes from significant results of the comparisons PE vs. Term norm and RPL vs. 1st term. Marked in red are the five genes that were significant in both studies (HIST1H4F, HIST2H2AC, HIST1H2AE, GRINA and PIGT). Log2 fold change was positive if the gene was upregulated in the complicated group compared to the respective normal group.

Furthermore, we explored the overall correlation in fold-change values among all 399 significant genes. Although the Pearson correlation P value was significant ($P = 5.3 \times 10^{-14}$), the correlation was relatively weak (R2 = 0.14) and slightly negative (Figure 9). From this, we could infer that the placental response to RPL and PE involves some degree of inverse relationship. However, a gene set enrichment analysis revealed limited overlap between the two groups. The only common matches were "extracellular exosome" (GO:0070062) and its higher level counterparts "extracellular vesicle" (GO:1903561), extracellular organelle (GO:0065010), and extracellular membrane-bounded organelle (GO:0043230), which were associated with 24% (n = 25) of the RPL genes and 19% (n = 33) of PE genes. When comparing the gene set enrichment results of up- and downregulated genes in RPL and PE, it was notable that RPL was enriched for several cell death and apoptosis-related categories. For example, genes upregulated in RPL were enriched for "programmed cell death" (GO:0012501, n = 18), "regulation of programmed cell death" (GO:0043067, n = 10) and "apoptotic process" (n = 15). Genes downregulated in RPL were enriched for "Diseases of programmed cell death" (REAC:R-HSA-9645723, n = 10) and "Apoptosis induced DNA fragmentation" (REAC:R-HSA-140342, n = 4). No categories directly related to cell death or apoptosis were found among the gene sets enriched in LO-PE.

4.3.5. Take home message: placental differential expression in pregnancy complications

The study showed that the placental transcriptome profile of LO-PE is clearly distinguishable from normal pregnancies (215 significantly affected genes) and other gestational complications. Other analyzed late-term complications (GD, SGA, LGA) did not present major differences in the placental transcriptome compared to uncomplicated gestations. Also, chorionic villous samples from the major pregnancy complication of the 1st trimester, RPL, showed a substantial number of differentially expressed genes (189). However, the affected molecular and cellular pathways were vastly different from LO-PE. Only 5 loci overlapped in the two datasets of differentially expressed genes (*HIST1H4F, HIST2H2AC, HIST1H2AE, GRINA* and *PIGT*), and these showed opposite directions of gene expression alteration in LO-PE and RPL.

4.4. Parent-of-origin-specific allelic expression in the placenta (Study IV)

The study aimed to investigate the manifestation of genomic imprinting in the human placenta by analyzing the parent-of-origin of placental transcripts from 91 imprinting candidate genes proposed by previous studies. The study benefited from the high-quality RNA-seq dataset encompassing a broad range of pregnancy outcomes (LO-PE, GD, SGA, LGA; Study II) and gestational ages (Study II–III), as well as available parental genotype data. This allowed us to examine the profile of parent-of-origin allelic expression in the placenta with regards to gestational dynamics and assess the stability of imprinting in complicated pregnancies.

4.4.1. Parental monoallelic expression is limited to well-known placental imprinted genes

Among 91 candidate imprinted genes, only 11 (12.1%) were found to be expressed exclusively in a parent-of-origin manner and were classified as imprinted genes (with > 90% of transcripts originating from one parental allele) (Figure 10 A). Paternally expressed genes showed varying levels of imprinting, with *PEG10* being the most stringent and *AIM1* being the least conservative. Among maternal genes, *MEG3* had the highest constraint for parental monoallelic expression and *H19* had the lowest. Interestingly, more paternally expressed imprinted genes were identified than maternally expressed ones.

All but one of the high-confidence imprinted genes expressed in the placenta have been reported to be imprinted also in the mouse (Table 7). The confirmed imprinted genes are either placenta-specific or also expressed in the adrenal gland (Figure 10 B).

Importantly, none of the imprinted genes displayed any systematic parent-of-origin dependent expressional bias in the placentas of term cases of LO-PE, GD, and deliveries of SGA or LGA newborns.

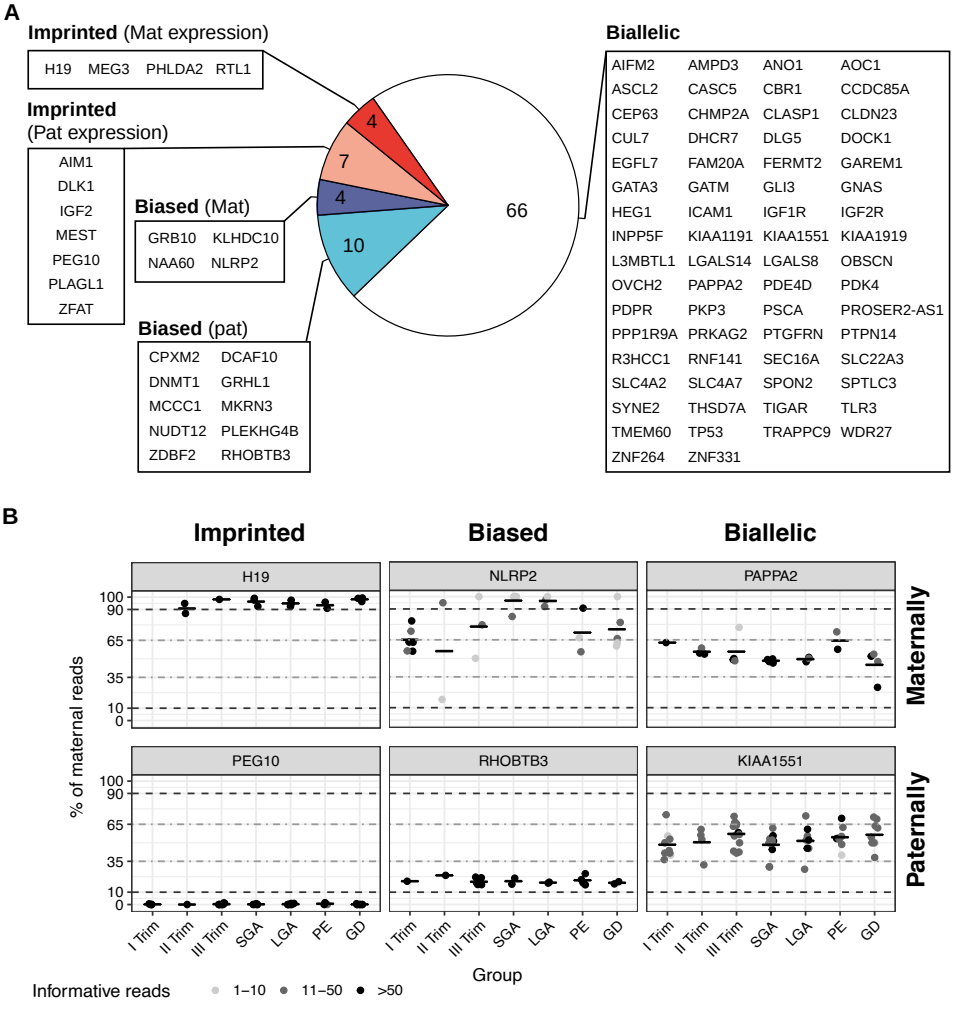

Figure 10: A) The analyzed gene set comprised 11 true imprinted genes, 14 genes with biased parental allelic expression, and 66 biallelic loci. B) Examples of imprinted, biased and biallelic gene expressions. Imprinted genes were expressed in a parent-of-origin-specific manner, with over 90% of transcripts originating from one parental allele. Biased parental allelic expression was observed when there was a significant deviation from equal proportions of transcribed parental alleles, but not exclusive monoallelic transcription. Biallelic expression was confirmed when the proportions of parental reads did not significantly differ from the expected ratio or when the estimated proportions of both parental allelic reads fall within 35–65%. GD – gestational diabetes; LGA – large-for-gestational-age newborn; PE – preeclampsia; SGA – small-for-gestational-age newborn; Trim – trimester. Adapted from Ref. IV.

Table 7: Genes that displayed imprinted allelic expression in our study.

Gene	Mean FPKM[a]	% mat reads (95% CI)	P value[b] (corr)	Class	Exp. allele	Impr in mouse[d]
MEG3	104.0	1 (0.99–1)	2.2×10^{-300}	Impr	Mat	Yes
PHLDA2	5.9	0.97 (0.94–0.99)	2.2×10^{-37}	Impr	Mat	Yes
RTL1	4.0	0.95 (0.94–0.96)	4.3×10^{-156}	Impr	Mat[e]	Yes
H19	498.0	0.94 (0.93–0.94)	4.7×10^{-322}	Impr	Mat	Yes
PEG10	102.1	0 (0–0)	2.2×10^{-300}	Impr	Pat	Yes
IGF2	84.5	0.01 (0.01–0.01)	2.2×10^{-300}	Impr	Pat	Yes
MEST	97.3	0.01 (0.01–0.02)	1.5×10^{-234}	Impr	Pat	Yes
ZFAT	101.8	0.01 (0.01–0.02)	4.7×10^{-322}	Impr	Pat	No
PLAGL1	22.7	0.02 (0.01–0.04)	6.5×10^{-103}	Impr	Pat	Yes
DLK1	54.9	0.03 (0.03–0.04)	2.2×10^{-300}	Impr	Pat	Yes
AIM1	55.6	0.06 (0.05–0.08)	4.3×10^{-206}	Impr	Pat	n.a.
KLHDC10	5.8	0.75 (0.71–0.79)	3.0×10^{-18}	Biased	Mat	n.a.
NLRP2	14.9	0.71 (0.69–0.74)	3.3×10^{-39}	Biased	Mat	n.a.
GRB10	9.9	0.67 (0.64–0.71)	1.3×10^{-11}	Biased	Mat	Yes (isoform)
NAA60	6.0	0.67 (0.61–0.72)	1.8×10^{-5}	Biased	Mat	n.a.
CPXM2	9.1	0.11 (0.09–0.13)	1.4×10^{-99}	Biased	Pat	n.a.
MCCC1	4.3	0.12 (0.08–0.18)	1.9×10^{-15}	Biased	Pat	n.a.
PLEKHG4B	0.9	0.15 (0.12–0.19)	1.1×10^{-39}	Biased	Pat	n.a.
DCAF10	4.2	0.16 (0.1–0.24)	1.9×10^{-9}	Biased	Pat	n.a.
DNMT1	8.9	0.17 (0.14–0.2)	4.5×10^{-44}	Biased	Pat	n.a.
NUDT12	2.1	0.17 (0.07–0.33)	5×10^{-2}	Biased	Pat	n.a.
RHOBTB3	358.0	0.18 (0.18–0.19)	4.7×10^{-322}	Biased	Pat	n.a.
ZDBF2	8.9	0.2 (0.18–0.23)	3.7×10^{-89}	Biased	Pat	Yes
MKRN3	3.5	0.3 (0.24–0.37)	1.7×10^{-4}	Biased	Pat	Yes
GRHL1	28.7	0.35 (0.34–0.36)	1.2×10^{-104}	Biased	Pat	n.a.

[a] Gene expression level across all analyzed placental samples, including the three trimesters of uncomplicated pregnancy and four clinical subgroups of complicated pregnancies
[b] The observed parental transcript ratios were statistically tested under the assumption that both alleles are expressed at equal levels, using binomial test implemented in R. Statistical significance level was defined $P < 0.05$ after application of Bonferroni correction for the number of conducted tests ($n = 91$)
[c] From (Court et al., 2014; Hamada et al., 2016; Metsalu et al., 2014)
[d] Data from Geneimprint database (Geneimprint : Genes, 2018)
[e] Maternal allelic expression was experimentally confirmed by RT-PCR, cloning, and sequencing
FPKM, fragments per kilobase of transcript per million mapped reads; impr,imprinted; Mat, maternal; Pat, paternal; SD, standard deviation; n.a., not available Modified from Ref. IV

4.4.2. The majority of candidate imprinted genes detected exhibit biallelic expression in the human placenta

Robust biallelic expression in the placenta was detected for 66 of 91 (72.5%) of the analyzed candidate imprinted genes (Figure 10 B), i.e. both parental alleles were equally transcribed. Most of these genes (92%) were broadly expressed across tissues (47 genes) or exhibited enhanced expression in organs other than the placenta (14 genes) (Fig N A). The transcript levels of biallelic placental genes varied, with some showing placenta-specific expression and others showing enhanced expression in other organs. However, when comparing the placental imprinted and biased genes, the average expression tended to be higher (imprinted (102.9 FPKM) vs. unimprinted (26.7 FPKM) single-tailed Welch Two Sample t-test P value = 0.049) (Figure 11 B).

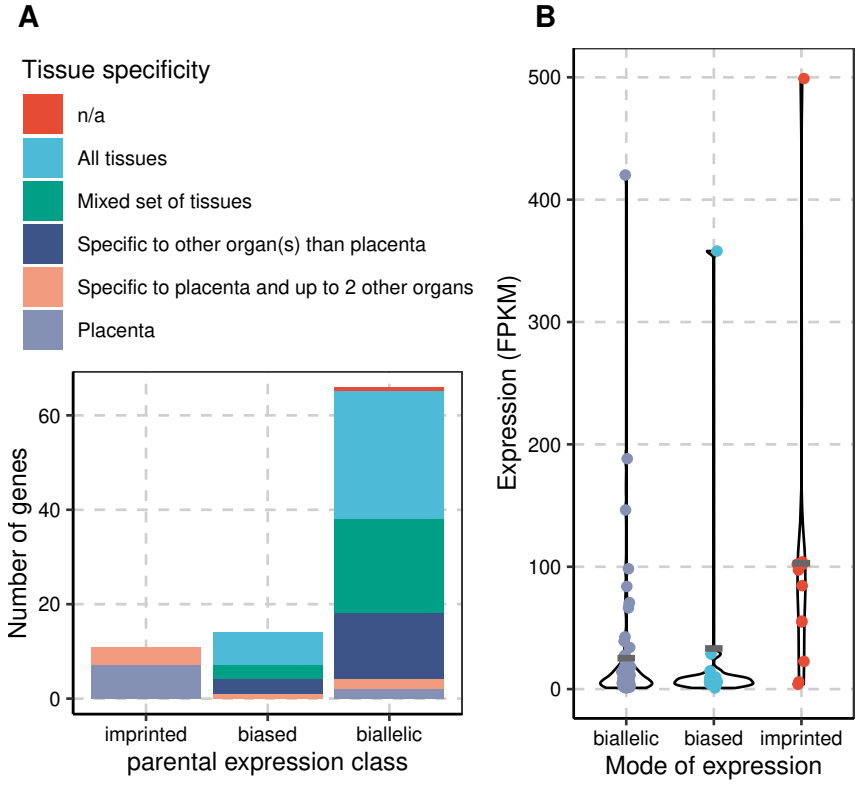

Figure 11: A) Expressional breadth across human tissues and B) violin plot of the average expressions (group mean is marked by grey crossbars) for the imprinted, biased and biallelic transcripts. Human tissue data was derived from the Protein Atlas database (Uhlén et al., 2015). FPKM, fragments per kilobase of transcript per million mapped reads. Modified from Ref. IV

4.4.3. A new class of placental loci – 14 genes exhibit biased parental allelic expression in the placenta

We have identified a group of 14 candidate imprinted genes (accounting for 15.4% of tested genes) with high confidence that exhibit biased allelic expression in the placenta, with 65–90% of reads coming from one parental allele (binomial test, $P_{corr} < 0.05$; Fig N B). Most of the genes with biased expression showed variability in the proportions of parental reads more similar to biallelic expression than to fully imprinted genes (Table 7, Figure 11). There were more genes with paternal bias (n = 10) than maternal bias (n = 4) and preferential transcription of the paternally biased genes was more pronounced compared to the maternally biased allele genes (median 83.0% vs. 69.3% of reads from the preferred parental allele, respectively). Although these candidate imprinted genes showed only biased, not exclusively monoallelic, parental allelic expression, the preferentially transcribed allele for all 14 genes was consistent with data from previous reports (Court et al., 2014; Geneimprint : Genes, 2018; Hamada et al., 2016; Metsalu et al., 2014). Notably, none of the genes with biased parental allelic expression were specific to the placenta, except for *MKRN3* (Figure 11 A). The placental expression level of these genes tended to be modest, except for *RHOBTB3* and *GRHL1* (Table 7; Figure 11 B). Finally, we did not detect systematic deviations from biased parental allelic expression in our dataset in the placentas representing term pregnancy complications.

4.4.4 Take home message from parent-of-origin-specific allelic expression in the placenta

Of the 91 genes, only 11 (12.1%) displayed confident imprinted gene expression, and all of them were either placenta-specific or additionally expressed only in the adrenal gland. Additional 14 genes displayed another type of parental allelic expression, wherein 65–90% of reads originated from one parental allele, we coined this mode of expression as biased. Over 72% of the proposed imprinted candidate genes showed confident biallelic expression in the human placenta (66 of 91 analyzed genes).

5. DISCUSSION

5.1. RNA integrity modulates RNA-Seq profile (Study I)

As the use of RNA-Seq continues to grow in dominance as a method of choice for transcriptome-wide gene expression quantification, there is considerable interest in understanding how the quality of input RNA affects the results of RNA-Seq experiments. Few previous studies have used deliberately degraded RNA samples over a wide range of RNA integrity values (ranging from highly degraded to virtually intact) in peripheral blood mononuclear cell samples (Gallego Romero et al., 2014) or have focused on brain tissue (Feng et al., 2015). The current study, in contrast, explores a real-life clinically collected sample set of human placental tissue and covers a more typical range of RIN values for clinical studies (RIN range: 6.1–8.2) (Aguet et al., 2020) where some RNA degradation is present.

Our results indicated that RNA integrity statistically affects expression level estimates for a large fraction of genes. Among all genes with adequate expression levels for analysis (mean read count ≥ 50 raw reads; n = 14,538), 8.1% showed statistically significant perturbations in transcript level estimates. However, examination of the quantile-quantile plot (Figure 5 A) indicates a much more pervasive effect, and the transcript level estimates of the majority of genes appear to be affected by RNA integrity. While these perturbations did not reach statistical significance in our study, the number of detected associations would increase with higher statistical power, i.e., a larger sample size and/or RIN range.

The detected fold changes of RIN-dependent alterations in transcript level estimates ranged from –7.58 to 2.89 per unit of RIN (Figure 5 B). The counter-intuitive phenomenon of genes exhibiting negative correlations to RIN, that is, higher expression at lower RIN values, can be explained mainly by data normalization. As RNAs are degraded at varying rates, more stable transcripts obtain higher relative concentrations than transcripts with fast degradation rates. After the data is normalized to equalize expression level distributions between samples, the stable transcripts attain elevated read counts in degraded samples.

Genes with the most extreme fold changes tend to have relatively low expression levels, and thus, the estimated fold changes were less reliable. Genes with the most statistically significant alterations demonstrated fold changes below 1.5. We hypothesize that most transcripts have inherent stability properties that determine their relative degradation rates and exhibit fold changes between 2.9 and –2.9 per unit of RIN.

The top five percent of genes with the strongest negative correlations to RIN include five members of the IGFBP family of insulin-like growth factor-binding proteins (*IGFBP1, IGFBP2, IGFBP4, IGFBP5, IGFBP7*) and four matrix metalloproteinase genes (*MMP1, MMP9, MMP12, MMP19*). We propose that such genes fall outside the typical spectrum of stability-driven degradation rates

and may be upregulated as a cause or a consequence of processes associated with increased RNA degradation. Indeed, the *IGFBP* genes have shown strong up-regulation accompanied by a six-fold increase in RNA half-life in response to hypoxia (Sugawara et al., 2000), this process appears to be mediated by several AUUUA sequences in their 3' UTRs (Gay & Babajko, 2000). Warm ischemia, which occurs between sample extraction and RNA isolation, is the primary factor that negatively affects RNA quality in clinical samples. During this state, changes in cellular metabolism occur as a response to the condition, followed by cell death and RNA degradation (Y. Ma et al., 2012). I propose that genes that exhibit exceptional responses to RNA integrity may indicate metabolic responses to warm ischemia and, therefore, require careful consideration and critical evaluation if detected in differential expression analyses.

Comparisons between genes with slow and fast degradation rates revealed that protein-coding transcripts (the most prevalent type of transcript in our dataset) were present in the smallest proportion (59%) among genes with faster-than-average degradation rates. Genes with the fastest degradation rates exhibited high fractions of pseudo- and noncoding genes. This differs from the results that Gallego Romero et al., 2014 obtained in their study. However, they used Poly-A enrichment for library preparation, which may be responsible for the discrepancy with our findings, as total RNA Sequencing captures a larger fraction of non-coding transcripts. Such differences highlight the importance of methodological details in estimating RNA degradation rates. We also observed that exon counts, overall length, 5' UTR and 3' UTR lengths are greater among transcripts with lower estimated stability, which Feng et al. (Feng et al., 2015) confirmed Duan et al., 2013 reported similar results for transcript length, 5' and 3' UTR lengths. These robustly confirmed findings indicate that genes under stricter expression level control (potentially high functional importance) tend to have elevated degradation rates and, hence are most affected by RNA quality.

It has been established that RNA degradation does not affect all transcripts equally (Gallego Romero et al., 2014). Consequently, the standard normalization methods used to adjust for variations in sequencing depth between samples do not account for RNA integrity effects. The prevalent method for dealing with RNA integrity in gene expression studies involves imposing a RIN cutoff on the included samples. Such cutoffs, however, are study-specific, somewhat arbitrary, and tend to be influenced by practical considerations like sample availability, especially for rare, valuable, or legacy samples. Furthermore, as there is evidence that antemortem events may influence sample RNA quality (Durrenberger et al., 2010), applying RIN-based cutoffs may impair the capture of the complete biological variation of the condition under investigation. It has been shown that mitigating RNA integrity effects is possible by including the RIN values as a covariate in the regression models used for testing differential expression (Gallego Romero et al., 2014). This can improve both type 1 and type 2 error rates. However, the cost of including an additional parameter into the model may be significant for studies with small sample sizes that are common for RNA-Seq due to cost and difficulty obtaining samples.

Based on this study's results, RNA integrity is a critical confounder in RNA-Seq-based expression level estimation, even for studies employing ribodepletion in favour of poly-A enrichment. While most genes are affected in a minor way, a subset of transcripts can exhibit extreme alterations in transcript level estimates, even with slight differences in RNA integrity. However, biologically meaningful analyses can still be conducted on degraded RNA samples despite the reduced signal-to-noise ratio. I concur with Gallego Romero et al., 2014 that universal RNA integrity cutoffs cannot be meaningfully established and believe that the stringent RNA quality standards established for in vitro studies could deter researchers from reporting RIN values (which is critical and required for cross-study comparisons) or even conducting studies in the first place. If appropriate attention is given to minimizing intergroup differences in RNA quality, and if the differential expression results are interpreted with awareness of potential confounding effects of RNA quality, it is possible to avoid type I errors and elucidate biological differences in gene expression even using degraded RNA samples.

5.2. Placental differential gene expression in pregnancy complications – cause or consequence? (Study II–III)

This study aimed to describe the human placental transcriptome and investigate its link to a set of pregnancy complications (LO-PE, GD, LGA, SGA, RPL). Our results show that placental gene expression in LO-PE differs significantly from that in other term complications (Figure 6). From term samples, we report 215 differentially expressed genes in the case of PE, four in GD, two in SGA, and one for LGA. This suggests that distinct molecular mechanisms underlie the pathology of PE compared to other pregnancy-related conditions.

However, when checking the correlation between the gene expression fold changes (Figure 7 A), all term complications displayed positive, statistically significant correlations between each other. That was especially true in the cases of PE, LGA, and SGA, while GD showed a weaker but still statistically important correlation. This was further corroborated by checking for overlap between the top 200 genes from each complication (Figure 7 B). From this, the placental reaction to LGA, SGA, and LO-PE seems similar. In all cases, the placenta will face a bottleneck in its capacity to support the fetus and, as a response, activates pathways to increase its overall throughput. However, in the case of PE, the reaction will be excessive. GD differed the least from the Term norm group among the term pregnancy complications, indicating other causes may be responsible for this condition beyond altered placental gene expression.

Furthermore, we identified 189 differentially expressed genes in first-trimester RPL samples. The high number of DEGs, compared to other pregnancy complications (Figure 6 A), is noteworthy because we only had two samples in the RPL case group, significantly reducing our power to detect a statistically significant result. This highlights the extreme state of the placental transcriptome in the case of RPL. Most DEGs (n = 138) were downregulated and associated with

transcriptional, telomere, spliceosome, cellular protein traffic complexes, chromatin assembly, and mitochondrial function (Figure 8 B). These processes are widespread and crucial for establishing a viable pregnancy, indicating that an underlying cause likely triggers the RPL condition, and the detected gene expression changes represent the outcome of this process. In addition to the referenced articles, as part of this thesis, I also compared the differentially expressed genes between RPL and our most severe term complication, LO-PE. Only five significant genes were shared between the two studies, but their fold changes were opposite, as was the overall correlation of the respective studies' gene expression changes (Figure 9). While LO-PE, SGA and LGA were at least conceptually aiming to support the fetus, and their gene expressions were positively correlated with each other, the transcriptome of RPL is inverse. This and the enrichment of several cell death-related pathways among the RPL DEGs further support the theory that the gene expression observed in RPL is, at that point, no longer trying to support the pregnancy but to terminate it.

5.3. Variable degree of parent-of-origin-specific allelic expression in the placenta – functional and evolutionary adaptation? (Study IV)

This study offers a systematic evaluation of parental allelic expression of nearly 400 candidate imprinted genes in 54 human placental samples, across all three trimesters of normal gestation, as well as in cases of LO-PE, GD, LGA and SGA. The study filtered the initial gene list for the presence of common exonic SNPs, sequencing depth, and informative families for the parental allelic expression, ultimately retaining a total of 91 genes for the final analysis. The findings revealed that only 11 of the 91 analyzed genes (12.1%) showed confident signals of parent-of-origin-specific allelic expression in the human placenta. Moreover, the programming of imprinting was stable across the entire gestation and assessed term pregnancy scenarios. The results suggested that the placenta-specific imprinted genes play a critical role in supporting the fine-tuning of developmental programming, as evidenced by their high expression in the second trimester of pregnancy. The study also identified a distinct class of 14 genes that showed a systematic bias towards the enrichment of transcripts from one parental allele, suggesting that the molecular mechanisms leading to biased parental allelic expression require further investigation. Overall, the study's results suggest that a restricted number of imprinted genes in the human placenta is consistent with the data on the mouse placenta and other human tissues. The study's findings may have implications for understanding the epigenetic regulation of fetal development and the aetiology of pregnancy complications.

5.4. Study limitations and perspectives

Recognizing the inherent limitations faced by placental gene expression studies investigating the causes and mechanisms of human pregnancy complications is crucial. These limitations are influenced by ethical considerations, the rapid evolution of the placenta, the complexity of the disorders, and inadequate funding, all of which highlight the challenges in this field. Consequently, sample sizes are often small (e.g., in our RPL group), or case-control groups have systematic differences (e.g., different gestational ages between PE and normal term samples). This thesis only used bulk RNA-Seq. Since the placenta has multiple cell types, higher resolution methods such as single-cell RNA-Seq or spatial transcriptomics could provide additional insights about normal and complicated pregnancies (Bhalla et al., 2023; Garcia-Flores et al., 2024; Greenbaum et al., 2023; Smith et al., 2024).

Also, due to the high risk for maternal and fetal health, placental sampling is mostly impossible before delivery or pregnancy termination. This limits us to a snapshot of the case after the effect, and it becomes more challenging to isolate the genes and pathways that drive the complications and could serve as a target for preventative treatment. In the case of RPL placentae, there was an additional confounding effect in that we did not precisely know for how long the fetus was deceased.

One potential way to address this issue would be to conduct such experiments on animal models. However, the rapid evolution of the placenta has limited the suitability of animal models for studying complex human placental diseases, as most do not fully replicate the spectrum of symptoms seen in human pathology (Chaouat & Clark, 2015; Chau et al., 2022; Sunderland et al., 2011). For instance, while there are numerous animal models for preeclampsia that have provided valuable insights and intervention strategies for this complex disease, translating these findings to clinical practice is yet to be realized (Chau et al., 2022; Taylor & George, 2022) Nevertheless, I believe that at this point, the term complications addressed in this thesis have been studied to a sufficient degree in a manner similar to ours, and the way forward is to study specific pathways and aetiological variations in animal models.

As for RPL, similar experiments would likely still need to be conducted, but with a significantly larger sample size, and if possible, by including placental samples from elective terminations from couples with an existing RPL diagnosis.

The current literature on biased parental expression of placental genes seems to be quite limited, and therefore, research in this field is likely to provide many opportunities for identifying novel mechanisms and affected genes.

6. CONCLUSIONS

Placental transcriptome research utilizing advanced tools like RNA-Seq offers a high throughput and comprehensive dataset, enabling a deeper understanding of the complex mechanisms involved in pregnancy complications. Previously available gene expression profiling methods did not provide similar throughput and accuracy to analyze the entire transcriptome cost-effectively. At the start of this doctoral project, there were limited publications about the methods to generate and analyze placental RNA-Seq datasets. This research contributed to the field by clarifying the adequate steps for designing a high-quality placental RNA-Seq study and provided candidate molecular mechanisms/pathways to understand placental malfunction in pregnancy disorders that could contextualize further investigations.

The main conclusions to be drawn from this thesis are:

1) Even minor differences in RNA integrity can lead to biased estimates of gene expression levels and RNA molecule's physical and biological parameters correlate with the strength of the effect. It is inadvisable to design studies with significantly different RNA integrity levels between clinical groups, as it will be difficult to distinguish the effects of RNA degradation from genuine differences in expression due to clinical or biological factors. Furthermore, even when groups exhibit similar average RNA degradation levels, a wider variance in RNA integrity can reduce the ability to identify differentially expressed genes due to increased noise.
2) The distinct transcriptome profile of placental samples from LO-PE pregnancies compared to normal pregnancies and other gestational complications provides evidence for the placental origin of this condition. At the same time, there was a statistically significant overlap among all term complication gene profiles. LO-PE and SGA have the most significant similarities, hinting at shared aetiology or placental reaction to the conditions.
3) RPL exhibited a major reorganization of the placental transcriptome compared to the 1st TRIM transcriptome. Closer inspection of the differentially expressed genes ontology indicated that the placental tissue was already winding down the cellular machinery and terminating the pregnancy at the time of sampling. The likely root cause for the RPL condition was not identified.
4) Most of the tested imprinting candidate genes showed biallelic gene expression in the human placenta. Only 11 genes (12.1% of tested genes) showed robust parent-of-origin-determined monoallelic gene expression. These imprinted genes were either placenta-specific or were transcribed additionally to the placenta only in the adrenal gland. Further 14 genes displayed parental biased expression, wherein 65–90% of RNA-seq reads originated from one parental allele.

SUMMARY IN ESTONIAN

Platsenta transkriptoom normaalse ja komplitseeritud raseduste korral

Rasedus on keeruline protsess, mille käigus areneb viljastatud munarakust laps. Tavaliselt kestab rasedus umbes 40 nädalat, mis on ajaliselt jasotatud kolmeks trimestriks. Raseduse ajal toimub ema kehas palju muudatusi, et toetada arenevat loodet ja platsenta moodustumist. Platsenta on ainulaadne organ, mis eksisteerb vaid ajutiselt. Platsenta moodustub samadest rakkudest, mis moodustavad embrüo ning see on nabanööri kaudu tihedalt seotud lootega. Platsenta mängib raseduse ajal üliolulist rolli, kuna see varustab kasvavat loodet hapniku ja toitainetega, eemaldades samal ajal jääkaineid loote verest. Lisaks on sellel ka endokriinne funktsioon, mis aitab emal rasedusega kohaneda ja seda säilitada.

Kahjuks ei kulge iga rasedus probleemideta. Statistika näitab, et ligikaudu 7–21% rasedusi mõjutavad hüpertensiivsed häired (Umesawa & Kobashi, 2016) ja 1–5% paaridest kogevad korduvat raseduse katkemist (Jauniaux et al., 2006; Regan et al., 2023). Implantatsioonihäired, ebanormaalne platsenta areng ja platsenta talitlushäired kujutavad endast suurt riskifaktorit raseduskomplikatsioonide, nagu raseduse katkemine, preeklampsia (PE), rasedusdiabeet (GD), loote kasvu peatumise või enneaegse sünnituse tekkeks (Sõber et al., 2015). Varasemad uuringud on veenvalt tuvastanud seose raseduse tüsistuste ja normaalist kõrvalekalduva platsenta geeniekspressiooni vahel. Seetõttu võib platsenta transkriptoomi uurimine anda väärtuslikku teavet nende seisundite diagnoosimiseks, ennetamiseks ja raviks. Selle doktoriprojekti alguses oli RNA-Seq (transkriptoomi sekveneerimine) uusim ja täpseim meetod, et korraga analüüsida kogu transkriptoomi. Varem kättesaadavad geeniekspressiooni profiilide koostamise meetodid ei andnud sarnast läbilaskevõimet (nt RT-qPCR) või täpsust (nt geeniekspressiooni geenikiibid) kogu transkriptoomi kuluefektiivseks analüüsimiseks.

Käesoleva doktoritöö käigus sekveneerisime suurima selleks hetkeks avaldatud raseduskomplikatsioone käsitleva platsenta RNA-Seq andmestiku. Andmekogum sisaldas kliiniliselt normaalsete raseduste platsentasid kõigist kolmest trimestrist (1st TRIM, 2nd TRIM ja Term norm), nelja raseduskomplikatsiooni valimeid kolmandast trimestrist (gestatsioonidiabeet (GD), preeklampsia (PE), väikese (SGA) ja suure sünnikaaluga rasedused (LGA)) ning ühte esimese trimestri korduva resedus katkemise (RPL) gruppi.

Tagamaks andmestiku kõrge kvaliteeti, analüüsisin, mil määral võib RNA lagunemine järgnevate katsete tulemusi mõjutada. Edasi leidsime, millised geenid ja rajad ekspresseeruvad erinevalt raseduskomplikatsioonide korral ning uurisime seost raseduse tüsistuste ja platsenta geenide vanemliku alleelse ekspressiooni vahel. Selle doktoritöö põhieesmärk oli välja töötada eksperimentaalne ning analüütiline protsess platsenta RNA-Seq andmestiku genereerimiseks ja sealt leida, mis geenid ja protsessid on raseduskomplikatsioonide korral häiritud.

Lõputööks sõnastati kolm järgmist eesmärki:

1. Hinnata platsentas pärast sünnitust esineva RNA lagunemise mõju RNA-Seq eksperimendi tulemustele.
2. Iseloomustada varaste ja hiliste raseduskomplikatsioonide puhul platsentas diferentsiaalse ekspressiooniga geenide ja kaasatud bioloogiliste radade maastikku.
3. Määrata 91 vermingu kandidaatgeeni vanemliku alleelse ekspressiooni hälvet platsentas ja analüüsida nende transkriptsiooni dünaamikat raseduse kulu ja komplikatsioonide kontekstis.

Nende eesmärkide täitmise raames osalesin ka nelja teadusartikli koostamises.

Peamised järeldused on järgmised:

1. Isegi väikesed erinevused RNA intaktsuses võivad viia geenieksprессioonitasemete muutuseni, kusjuures RNA molekuli füüsilised ja bioloogilised parameetrid korreleeruvad selle mõju määraga. Ei ole mõistlik kavandada eksperimente, kus erinevate kliiniliste gruppide vahel on oluline erinevus RNA intaktuses, sest muidu on hiljem keeruline eristada RNA lagunemise mõju gruppide vahel esinevast bioloogilisest mõjust. Isegi kui gruppidel on samad keskmised RNA lagunemise tasemed, võib RNA terviklikkuse suurem varieeruvus siiski langetada võimet tuvastada erinevalt ekspresseeritud geene.
2. Preklamptiliste raseduste puhul on platsenta transkriptoom selgelt erinev normaalist, kinnitades teooriat, et preeklampsia on platsentast tulenev komplikatsioon. Kuigi teised raseduse hilisema osa komplikatsioonid olid statistiliselt oluliselt normaalist diferentsiaalses ekspressioonis vaid mõne geeniga, oli kõikidel hilise raseduskomplikatsioonide platsenta transkriptoomide profiilidel omavahel statistiliselt oluline positiivne korrelatsioon. LO-PE ja SAG grupp olid omavahel kõige sarnasemad, viidates nende komplikatsioonide etioloogilisele sarnasusele.
3. RPL platsenta transkriptoomi oli 1. TRIM-i transkriptoomiga võrreldes oluliselt teistsugusem. Diferentsiaalselt ekspresseeritud geenide ontoloogia lähem uurimine viitas, et platsenta kude oli proovide võtmise ajaks juba rakusurma ja raseduse katkestamise rajal ning juurpõhjust RPL tekkeks sealt eristada ei õnnestunud.
4. Enamik testitud vermingu kandidaatgeene ei olnud inimese platsentas vermitud. Ainult 11 geenil (12,1% testitud geenidest) ilmnes tugev vanema poolt määratud monoalleelse geeni ekspressioon. Vermitud geenid olid kas platsentaspetsiifilised või transkribeeriti neid lisaks platsentale ainult neerupealistes. Veel 14 geenil oli kallutatud ekspressioon, mille puhul 65–90% RNA-seq lugemistest pärines ühest vanemalleelist.

REFERENCES

Addo, K. A., Palakodety, N., Hartwell, H. J., Tingare, A., & Fry, R. C. (2020). Placental microRNAs: Responders to environmental chemicals and mediators of pathophysiology of the human placenta. *Toxicology Reports*, *7*, 1046–1056. https://doi.org/10.1016/J.TOXREP.2020.08.002

Adobe Illustrator – valdkonna juhtiv vektorgraafikatarkvara. (n.d.). Retrieved July 30, 2024, from https://www.adobe.com/ee/products/illustrator.html

Aguet, F., Barbeira, A. N., Bonazzola, R., Brown, A., Castel, S. E., Jo, B., Kasela, S., Kim-Hellmuth, S., Liang, Y., Oliva, M., Flynn, E. D., Parsana, P., Fresard, L., Gamazon, E. R., Hamel, A. R., He, Y., Hormozdiari, F., Mohammadi, P., Muñoz-Aguirre, M., … Volpi, S. (2020). The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science*, *369*(6509), 1318–1330. https://doi.org/10.1126/SCIENCE.AAZ1776/SUPPL_FILE/AAZ1776_TABLESS10-S16.XLSX

Ahmed, B., Abushama, M., & Konje, J. C. (2023). Prevention of spontaneous preterm delivery – an update on where we are today. *The Journal of Maternal-Fetal & Neonatal Medicine*, *36*(1). https://doi.org/10.1080/14767058.2023.2183756

Akre, S., Sharma, K., Chakole, S., & Wanjari, M. B. (2022). Eclampsia and Its Treatment Modalities: A Review Article. *Cureus*, *14*(9). https://doi.org/10.7759/CUREUS.29080

Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biology*, *11*(10), 1–12. https://doi.org/10.1186/GB-2010-11-10-R106/COMMENTS

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169. https://doi.org/10.1093/BIOINFORMATICS/BTU638

Angiolini, E., Sandovici, I., Coan, P. M., Burton, G. J., Sibley, C. P., Fowden, A. L., & Constância, M. (2021). Deletion of the imprinted phlda2 gene increases placental passive permeability in the mouse. *Genes*, *12*(5). https://doi.org/10.3390/GENES12050639/S1

Armengaud, J. B., Yzydorczyk, C., Siddeek, B., Peyter, A. C., & Simeoni, U. (2021). Intrauterine growth restriction: Clinical consequences on health and disease at adulthood. *Reproductive Toxicology*, *99*, 168–176. https://doi.org/10.1016/J.REPROTOX.2020.10.005

Armstrong, D. L., McGowen, M. R., Weckle, A., Pantham, P., Caravas, J., Agnew, D., Benirschke, K., Savage-Rumbaugh, S., Nevo, E., Kim, C. J., Wagner, G. P., Romero, R., & Wildman, D. E. (2017). The core transcriptome of mammalian placentas and the divergence of expression with placental shape. *Placenta*, *57*, 71–78. https://doi.org/10.1016/J.PLACENTA.2017.04.015

Bhalla, N., Franzén, L., Scheynius, A., Papadogiannakis, N., Hansson, S. R., Lager, S., & Ståhl, P. L. (2023). Spatial transcriptomics of human placentas reveal distinct RNA patterns associated with morphology and preeclampsia. *Placenta*, *139*, 213–216. https://doi.org/10.1016/J.PLACENTA.2023.07.004

Boyd, H. A., Tahir, H., Wohlfahrt, J., & Melbye, M. (2013a). Associations of Personal and Family Preeclampsia History With the Risk of Early-, Intermediate- and Late-Onset Preeclampsia. *American Journal of Epidemiology*, *178*(11), 1611–1619. https://doi.org/10.1093/AJE/KWT189

Boyd, H. A., Tahir, H., Wohlfahrt, J., & Melbye, M. (2013b). Associations of Personal and Family Preeclampsia History With the Risk of Early-, Intermediate- and Late-Onset Preeclampsia. *American Journal of Epidemiology*, *178*(11), 1611–1619. https://doi.org/10.1093/AJE/KWT189

Bricker, L., & Farquharson, R. G. (2002). Types of pregnancy loss in recurrent miscarriage: implications for research and clinical practice. *Human Reproduction*, *17*(5), 1345–1350. https://doi.org/10.1093/HUMREP/17.5.1345

Burton, G. J., Sebire, N. J., Myatt, L., Tannetta, D., Wang, Y. L., Sadovsky, Y., Staff, A. C., & Redman, C. W. (2014). Optimising sample collection for placental research. *Placenta*, *35*(1), 9–22. https://doi.org/10.1016/J.PLACENTA.2013.11.005

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/CLINCHEM.2008.112797

Carr, D. B., Epplein, M., Johnson, C. O., Easterling, T. R., & Critchlow, C. W. (2005). A sister's risk: Family history as a predictor of preeclampsia. *American Journal of Obstetrics and Gynecology*, *193*(3), 965–972. https://doi.org/10.1016/J.AJOG.2005.06.034

Chaouat, G., & Clark, D. A. (2015). Are animal models useful or confusing in understanding the human feto-maternal relationship? A debate. *Journal of Reproductive Immunology*, *108*, 56–64. https://doi.org/10.1016/J.JRI.2014.10.004

Chau, K., Welsh, M., Makris, A., & Hennessy, A. (2022). Progress in preeclampsia: the contribution of animal models. *Journal of Human Hypertension*, *36*(8), 705. https://doi.org/10.1038/S41371-021-00637-X

Conesa, A., Madrigal, P., Tarazona, S., Gomez-Cabrero, D., Cervera, A., McPherson, A., Szcześniak, M. W., Gaffney, D. J., Elo, L. L., Zhang, X., & Mortazavi, A. (2016). A survey of best practices for RNA-seq data analysis. *Genome Biology*, *17*(1), 13. https://doi.org/10.1186/S13059-016-0881-8

Court, F., Tayama, C., Romanelli, V., Martin-Trujillo, A., Iglesias-Platas, I., Okamura, K., Sugahara, N., Simón, C., Moore, H., Harness, J. V., Keirstead, H., Sanchez-Mut, J. V., Kaneki, E., Lapunzina, P., Soejima, H., Wake, N., Esteller, M., Ogata, T., Hata, K., … Monk, D. (2014). Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment. *Genome Research*, *24*(4), 554. https://doi.org/10.1101/GR.164913.113

Cox, B., Leavey, K., Nosi, U., Wong, F., & Kingdom, J. (2015). Placental transcriptome in development and pathology: expression, function, and methods of analysis. *American Journal of Obstetrics and Gynecology*, *213*(4), S138–S151. https://doi.org/10.1016/J.AJOG.2015.07.046

Duan, J., Shi, J., Ge, X., Dölken, L., Moy, W., He, D., Shi, S., Sanders, A. R., Ross, J., & Gejman, P. V. (2013). Genome-wide survey of interindividual differences of RNA stability in human lymphoblastoid cell lines. *Scientific Reports 2013 3:1*, *3*(1), 1–10. https://doi.org/10.1038/srep01318

Duley, L. (2009). The Global Impact of Pre-eclampsia and Eclampsia. *Seminars in Perinatology*, *33*(3), 130–137. https://doi.org/10.1053/j.semperi.2009.02.010

Durrenberger, P. F., Fernando, S., Kashefi, S. N., Ferrer, I., Hauw, J. J., Seilhean, D., Smith, C., Walker, R., Al-Sarraj, S., Troakes, C., Palkovits, M., Kasztner, M., Huitinga, I., Arzberger, T., Dexter, D. T., Kretzschmar, H., & Reynolds, R. (2010).

Effects of antemortem and postmortem variables on human brain mRNA quality: a BrainNet Europe study. *Journal of Neuropathology and Experimental Neurology*, *69*(1), 70–81. https://doi.org/10.1097/NEN.0B013E3181C7E32F

Eades, C. E., Cameron, D. M., & Evans, J. M. M. (2017). Prevalence of gestational diabetes mellitus in Europe: A meta-analysis. *Diabetes Research and Clinical Practice*, *129*, 173–181. https://doi.org/10.1016/J.DIABRES.2017.03.030

Eaton, S. L., Roche, S. L., Llavero Hurtado, M., Oldknow, K. J., Farquharson, C., Gillingwater, T. H., & Wishart, T. M. (2013). Total protein analysis as a reliable loading control for quantitative fluorescent Western blotting. *PloS One*, *8*(8). https://doi.org/10.1371/JOURNAL.PONE.0072457

Eleje, G. U., Ugwu, E. O., Igbodike, E. P., Malachy, D. E., Nwankwo, E. U., Ugboaja, J. O., Ikechebelu, J. I., & Nwagha, U. I. (2023). Prevalence and associated factors of recurrent pregnancy loss in Nigeria according to different national and international criteria (ASRM/ESHRE vs. WHO/RCOG). *Frontiers in Reproductive Health*, *5*. https://doi.org/10.3389/FRPH.2023.1049711

Feng, H., Zhang, X., & Zhang, C. (2015). mRIN for direct assessment of genome-wide and gene-specific mRNA integrity from large-scale RNA-sequencing data. *Nature Communications 2015 6:1*, *6*(1), 1–10. https://doi.org/10.1038/ncomms8816

Fowden, A. L., Forhead, A. J., Sferruzzi-Perri, A. N., Burton, G. J., & Vaughan, O. R. (2015). Review: Endocrine regulation of placental phenotype. *Placenta*, *36 Suppl 1*(S1), S50–S59. https://doi.org/10.1016/J.PLACENTA.2014.11.018

Gallego Romero, I., Pai, A. A., Tung, J., & Gilad, Y. (2014). RNA-seq: Impact of RNA degradation on transcript quantification. *BMC Biology*, *12*(1), 1–13. https://doi.org/10.1186/1741-7007-12-42/TABLES/3

Garcia-Flores, V., Romero, R., Tarca, A. L., Peyvandipour, A., Xu, Y., Galaz, J., Miller, D., Chaiworapongsa, T., Chaemsaithong, P., Berry, S. M., Awonuga, A. O., Bryant, D. R., Pique-Regi, R., & Gomez-Lopez, N. (2024). Deciphering maternal-fetal crosstalk in the human placenta during parturition using single-cell RNA sequencing. *Science Translational Medicine*, *16*(729), eadh8335. https://doi.org/10.1126/SCITRANSLMED.ADH8335

Gay, E., & Babajko, S. (2000). AUUUA sequences compromise human insulin-like growth factor binding protein-1 mRNA stability. *Biochemical and Biophysical Research Communications*, *267*(2), 509–515. https://doi.org/10.1006/BBRC.1999.2000

Geneimprint : Genes. (n.d.). Retrieved May 25, 2018, from https://www.geneimprint.org/site/genes-by-species

Gheorghe, C. P., Goyal, R., Mittal, A., & Longo, L. D. (2010). Gene expression in the placenta: maternal stress and epigenetic responses. *The International Journal of Developmental Biology*, *54*(2–3), 507–523. https://doi.org/10.1387/IJDB.082770CG

Gong, S., Gaccioli, F., Aye, I. L. M. H., Avellino, G., Cook, E., Lawson, A. R. J., Harvey, L. M. R., Smith, G. C. S., & Charnock-Jones, D. S. (2023). The human placenta exhibits a unique transcriptomic void. *Cell Reports*, *42*(7), 112800. https://doi.org/10.1016/J.CELREP.2023.112800

Gong, X., He, W., Jin, W., Ma, H., Wang, G., Li, J., Xiao, Y., Zhao, Y., Chen, Q., Guo, H., Yang, J., Qi, Y., Dong, W., Fu, M., Li, X., Liu, J., Liu, X., Yin, A., Zhang, Y., & Wei, Y. (2024). Disruption of maternal vascular remodeling by a fetal endoretrovirus-derived gene in preeclampsia. *Genome Biology*, *25*(1), 117. https://doi.org/10.1186/S13059-024-03265-Z

Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews. Genetics*, *17*(6), 333. https://doi.org/10.1038/NRG.2016.49

Greenbaum, S., Averbukh, I., Soon, E., Rizzuto, G., Baranski, A., Greenwald, N. F., Kagel, A., Bosse, M., Jaswa, E. G., Khair, Z., Kwok, S., Warshawsky, S., Piyadasa, H., Goldston, M., Spence, A., Miller, G., Schwartz, M., Graf, W., Van Valen, D., … Angelo, M. (2023). A spatially resolved timeline of the human maternal–fetal interface. *Nature*, *619*(7970), 595. https://doi.org/10.1038/S41586-023-06298-9

Gude, N. M., Roberts, C. T., Kalionis, B., & King, R. G. (2004). Growth and function of the normal human placenta. *Thrombosis Research*, *114*(5–6), 397–407. https://doi.org/10.1016/J.THROMRES.2004.06.038

Hamada, H., Okae, H., Toh, H., Chiba, H., Hiura, H., Shirane, K., Sato, T., Suyama, M., Yaegashi, N., Sasaki, H., & Arima, T. (2016). Allele-Specific Methylome and Transcriptome Analysis Reveals Widespread Imprinting in the Human Placenta. *American Journal of Human Genetics*, *99*(5), 1045–1058. https://doi.org/10.1016/J.AJHG.2016.08.021

Head, S. R., Kiyomi Komori, H., LaMere, S. A., Whisenant, T., Van Nieuwerburgh, F., Salomon, D. R., & Ordoukhanian, P. (2014). Library construction for next-generation sequencing: Overviews and challenges. *BioTechniques*, *56*(2), 61. https://doi.org/10.2144/000114133

Hill, M. (2010). *The Carnegie Staged Embryos*. 84. https://embryology.med.unsw.edu.au/embryology/images/5/56/The_Carnegie_Staged_Embryos.pdf

Hu, X. Q., & Zhang, L. (2019). MicroRNAs in Uteroplacental Vascular Dysfunction. *Cells*, *8*(11). https://doi.org/10.3390/CELLS8111344

Hyun, I., Munsie, M., Pera, M. F., Rivron, N. C., & Rossant, J. (2020). Toward Guidelines for Research on Human Embryo Models Formed from Stem Cells. *Stem Cell Reports*, *14*(2), 169. https://doi.org/10.1016/J.STEMCR.2019.12.008

Inno, R., Kikas, T., Lillepea, K., & Laan, M. (2021). Coordinated Expressional Landscape of the Human Placental miRNome and Transcriptome. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/FCELL.2021.697947/FULL

Islam, S. M. R., Saha, S. K., El-Sappagh, S., Tariq, F., Das, J., Afzal, M., & Cho, S.-G. (2021). Expression of GRINA Correlates with Prognosis in Human Cancers: A Pan-cancer Analysis. *BioRxiv*, 2021.05.13.444089. https://doi.org/10.1101/2021.05.13.444089

Jauniaux, E., Farquharson, R. G., Christiansen, O. B., & Exalto, N. (2006a). Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Human Reproduction (Oxford, England)*, *21*(9), 2216–2222. https://doi.org/10.1093/HUMREP/DEL150

Jauniaux, E., Farquharson, R. G., Christiansen, O. B., & Exalto, N. (2006b). Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Human Reproduction (Oxford, England)*, *21*(9), 2216–2222. https://doi.org/10.1093/HUMREP/DEL150

Jeyarajah, M. J., Bhattad, G. J., Kelly, R. D., Baines, K. J., Jaremek, A., Yang, F. H. P., Okae, H., Arima, T., Dumeaux, V., & Renaud, S. J. (2022). The multifaceted role of GCM1 during trophoblast differentiation in the human placenta. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(49). https://doi.org/10.1073/PNAS.2203071119

Jukic, A. M., Baird, D. D., Weinberg, C. R., Mcconnaughey, D. R., & Wilcox, A. J. (2013). Length of human pregnancy and contributors to its natural variation. *Human Reproduction (Oxford, England)*, *28*(10), 2848. https://doi.org/10.1093/HUMREP/DET297

Kasak, L., Rull, K., & Laan, M. (2018). Genetics and genomics of recurrent pregnancy loss. *Human Reproductive and Prenatal Genetics*, 463–494. https://doi.org/10.1016/B978-0-12-813570-9.00021-8

Kasak, L., Rull, K., Sõber, S., & Laan, M. (2017). Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families. *Scientific Reports*, *7*. https://doi.org/10.1038/SREP45327

Kasak, L., Rull, K., Vaas, P., Teesalu, P., & Laan, M. (2015). Extensive load of somatic CNVs in the human placenta. *Scientific Reports*, *5*. https://doi.org/10.1038/SREP08342

Kersey, P. J., Allen, J. E., Armean, I., Boddu, S., Bolt, B. J., Carvalho-Silva, D., Christensen, M., Davis, P., Falin, L. J., Grabmueller, C., Humphrey, J., Kerhornou, A., Khobova, J., Aranganathan, N. K., Langridge, N., Lowy, E., McDowall, M. D., Maheswari, U., Nuhn, M., … Staines, D. M. (2016). Ensembl Genomes 2016: more genomes, more complexity. *Nucleic Acids Research*, *44*(D1), D574–D580. https://doi.org/10.1093/NAR/GKV1209

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., & Salzberg, S. L. (2013). TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology*, *14*(4), 1–13. https://doi.org/10.1186/GB-2013-14-4-R36/FIGURES/6

Kinsella, R. J., Kähäri, A., Haider, S., Zamora, J., Proctor, G., Spudich, G., Almeida-King, J., Staines, D., Derwent, P., Kerhornou, A., Kersey, P., & Flicek, P. (2011). Ensembl BioMarts: a hub for data retrieval across taxonomic space. *Database : The Journal of Biological Databases and Curation*, *2011*. https://doi.org/10.1093/DATABASE/BAR030

Kumar, A., Kankainen, M., Parsons, A., Kallioniemi, O., Mattila, P., & Heckman, C. A. (2017). The impact of RNA sequence library construction protocols on transcriptomic profiling of leukemia. *BMC Genomics*, *18*(1), 629. https://doi.org/10.1186/S12864-017-4039-1

Lai, C., Coulter, S. A., & Woodruff, A. (2017). Hypertension and Pregnancy. *Texas Heart Institute Journal*, *44*(5), 350. https://doi.org/10.14503/THIJ-17-6359

Lala, P. K., Nandi, P., Hadi, A., & Halari, C. (2021). A crossroad between placental and tumor biology: What have we learnt? *Placenta*, *116*, 12–30. https://doi.org/10.1016/J.PLACENTA.2021.03.003

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*. https://doi.org/10.1186/1471-2105-9-559

Larsen, E. C., Christiansen, O. B., Kolte, A. M., & Macklon, N. (2013). New insights into mechanisms behind miscarriage. *BMC Medicine*, *11*(1). https://doi.org/10.1186/1741-7015-11-154

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics (Oxford, England)*, *27*(21), 2987–2993. https://doi.org/10.1093/BIOINFORMATICS/BTR509

Lian, I. A., Løset, M., Mundal, S. B., Fenstad, M. H., Johnson, M. P., Eide, I. P., Bjørge, L., Freed, K. A., Moses, E. K., & Austgulen, R. (2011). Increased endoplasmic reticulum stress in decidual tissue from pregnancies complicated by fetal growth restriction with and without pre-eclampsia. *Placenta*, *32*(11), 823–829. https://doi.org/10.1016/J.PLACENTA.2011.08.005

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 1–21. https://doi.org/10.1186/S13059-014-0550-8/FIGURES/9

Lund, M., Kamper-Jørgensen, M., Nielsen, H. S., Lidegaard, Ø., Andersen, A. M. N., & Christiansen, O. B. (2012). Prognosis for live birth in women with recurrent miscarriage: What is the best measure of success? *Obstetrics and Gynecology*, *119*(1), 37–43. https://doi.org/10.1097/AOG.0B013E31823C0413

Ma, H., Zhang, X., Li, N., Lu, X., Wei, Y., Yuan, N., Tian, G., & Li, S. (2020). Glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1, a potential target of miR-296, facilitates proliferation and migration of rectal cancer cells. *Bioscience, Biotechnology, and Biochemistry*, *84*(10), 2077–2084. https://doi.org/10.1080/09168451.2020.1792267

Ma, Y., Dai, H., & Kong, X. (2012). Impact of warm ischemia on gene expression analysis in surgically removed biosamples. *Analytical Biochemistry*, *423*(2), 229–235. https://doi.org/10.1016/J.AB.2012.02.003

Männik, 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. *Molecular and Cellular Endocrinology*, *355*(1), 180. https://doi.org/10.1016/J.MCE.2012.02.009

Mazumder, T., Akter, E., Rahman, S. M., Islam, M. T., & Talukder, M. R. (2022). Prevalence and Risk Factors of Gestational Diabetes Mellitus in Bangladesh: Findings from Demographic Health Survey 2017–2018. *International Journal of Environmental Research and Public Health*, *19*(5), 2583. https://doi.org/10.3390/IJERPH19052583/S1

Metsalu, T., Viltrop, T., Tiirats, A., Rajashekar, B., Reimann, E., Kõks, S., Rull, K., Milani, L., Acharya, G., Basnet, P., Vilo, J., Mägi, R., Metspalu, A., Peters, M., Haller-Kikkatalo, K., & Salumets, A. (2014). Using RNA sequencing for identifying gene imprinting and random monoallelic expression in human placenta. *Epigenetics*, *9*(10), 1397–1409. https://doi.org/10.4161/15592294.2014.970052

Monk, D. (2015). Genomic imprinting in the human placenta. *American Journal of Obstetrics & Gynecology*, *213*(4), S152–S162. https://doi.org/10.1016/J.AJOG.2015.06.032

Moreira, M. E. L., & Méio, M. D. B. B. (2022). Small for Gestational Age. *Perinatology: Evidence-Based Best Practices in Perinatal Medicine*, 1151–1157. https://doi.org/10.1007/978-3-030-83434-0_59

Nagirnaja, L., Rull, K., Uusküla, L., Hallast, P., Grigorova, M., & Laan, M. (2010). Genomics and genetics of gonadotropin beta-subunit genes: Unique FSHB and duplicated LHB/CGB loci. *Molecular and Cellular Endocrinology*, *329*(1–2), 4. https://doi.org/10.1016/J.MCE.2010.04.024

Pal, A. K., Ambulkar, P. S., Waghmare, J. E., Wankhede, V., Shende, M. R., & Tarnekar, A. M. (2018). Chromosomal Aberrations in Couples with Pregnancy Loss: A Retrospective Study. *Journal of Human Reproductive Sciences*, *11*(3), 247. https://doi.org/10.4103/JHRS.JHRS_124_17

Panel, I. A. of D. and P. S. G. C. (2010). International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy. *Diabetes Care*, *33*(3), 676–682. https://doi.org/10.2337/DC09-1848

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon: fast and bias-aware quantification of transcript expression using dual-phase inference. *Nature Methods*, *14*(4), 417. https://doi.org/10.1038/NMETH.4197

Petriglia, G., Palaia, I., Musella, A., Marchetti, C., Antonilli, M., Brunelli, R., Ostuni, R., & Benedetti Panici, P. (2015). Threatened abortion and late-pregnancy complications: a case-control study and review of literature. *Minerva Ginecologica*, *67*(6), 491–497. https://pubmed.ncbi.nlm.nih.gov/25668506/

Placenta – Structure, Development, Functions and Facts. (n.d.). Retrieved July 31, 2024, from https://www.vedantu.com/biology/placenta

R Foundation for Statistical Computing, V. Austria. (n.d.). *R Core Team (2018). R: A language and environment for statistical computing.*

Rana, S., Lemoine, E., Granger, J., & Karumanchi, S. A. (2019). Preeclampsia: Pathophysiology, Challenges, and Perspectives. *Circulation Research*, *124*(7), 1094–1112. https://doi.org/10.1161/CIRCRESAHA.118.313276/ASSET/093CF6BB-9496-4C85-9583-5C41E1DFFFEA/ASSETS/IMAGES/LARGE/1094FIG03.JPG

Ratnik, K., Rull, K., Aasmets, O., Kikas, T., Hanson, E., Kisand, K., Fischer, K., & Laan, M. (2022). Novel Early Pregnancy Multimarker Screening Test for Preeclampsia Risk Prediction. *Frontiers in Cardiovascular Medicine*, *9*, 932480. https://doi.org/10.3389/FCVM.2022.932480/FULL

Regan, L., Rai, R., Saravelos, S., & Li, T. C. (2023a). Recurrent MiscarriageGreen-top Guideline No. 17. *BJOG: An International Journal of Obstetrics and Gynaecology*, *130*(12), e9–e39. https://doi.org/10.1111/1471-0528.17515

Regan, L., Rai, R., Saravelos, S., & Li, T. C. (2023b). Recurrent MiscarriageGreen-top Guideline No. 17. *BJOG: An International Journal of Obstetrics and Gynaecology*, *130*(12), e9–e39. https://doi.org/10.1111/1471-0528.17515

Reiman, M., Laan, M., Rull, K., & Sõber, S. (2017). Effects of RNA integrity on transcript quantification by total RNA sequencing of clinically collected human placental samples. *The FASEB Journal*, *31*(8), 3298–3308. https://doi.org/10.1096/FJ.201601031RR

Reimand, J., Kull, M., Peterson, H., Hansen, J., & Vilo, J. (2007). g:Profiler – a web-based toolset for functional profiling of gene lists from large-scale experiments. *Nucleic Acids Research*, *35*(suppl_2), W193–W200. https://doi.org/10.1093/NAR/GKM226

Roberts, J. M., & Escudero, C. (2012). The placenta in preeclampsia. *Pregnancy Hypertension*, *2*(2), 72. https://doi.org/10.1016/J.PREGHY.2012.01.001

Roberts, J. M., & Hubel, C. A. (2009). The Two Stage Model of Preeclampsia: Variations on the Theme. *Placenta*, *30*(SUPPL.), 32–37. https://doi.org/10.1016/J.PLACENTA.2008.11.009

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., & Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, *29*(1), 24–26. https://doi.org/10.1038/NBT.1754

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2009). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139. https://doi.org/10.1093/BIOINFORMATICS/BTP616

Rodriques, S. G., Stickels, R. R., Goeva, A., Martin, C. A., Murray, E., Vanderburg, C. R., Welch, J., Chen, L. M., Chen, F., & Macosko, E. Z. (2019). Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. *Science (New York, N.Y.)*, *363*(6434), 1463–1467. https://doi.org/10.1126/SCIENCE.AAW1219

Sandovici, I., Georgopoulou, A., Pérez-García, V., Hufnagel, A., López-Tello, J., Lam, B. Y. H., Schiefer, S. N., Gaudreau, C., Santos, F., Hoelle, K., Yeo, G. S. H., Burling, K., Reiterer, M., Fowden, A. L., Burton, G. J., Branco, C. M., Sferruzzi-Perri, A. N., & Constância, M. (2022). The imprinted Igf2-Igf2r axis is critical for matching placental microvasculature expansion to fetal growth. *Developmental Cell*, *57*(1), 63-79.e8. https://doi.org/10.1016/j.devcel.2021.12.005

Schroeder, A., Mueller, O., Stocker, S., Salowsky, R., Leiber, M., Gassmann, M., Lightfoot, S., Menzel, W., Granzow, M., & Ragg, T. (2006). The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Molecular Biology*, *7*, 3. https://doi.org/10.1186/1471-2199-7-3

Sharova, L. V., Sharov, A. A., Nedorezov, T., Piao, Y., Shaik, N., & Ko, M. S. H. (2009). Database for mRNA half-life of 19 977 genes obtained by DNA microarray analysis of pluripotent and differentiating mouse embryonic stem cells. *DNA Research : An International Journal for Rapid Publication of Reports on Genes and Genomes*, *16*(1), 45–58. https://doi.org/10.1093/DNARES/DSN030

Shu, X., Chen, N., Zhang, Y., Juan, J., Zheng, Y., & Yang, H. (2023). Fetal growth trajectories of small/large for gestational age infants in twin pregnancies. *American Journal of Obstetrics & Gynecology MFM*, *5*(8), 100999. https://doi.org/10.1016/J.AJOGMF.2023.100999

Sims, D., Sudbery, I., Ilott, N. E., Heger, A., & Ponting, C. P. (2014). Sequencing depth and coverage: key considerations in genomic analyses. *Nature Reviews. Genetics*, *15*(2), 121–132. https://doi.org/10.1038/NRG3642

Smith, K. D., Prince, D. K., MacDonald, J. W., Bammler, T. K., & Akilesh, S. (2024). Challenges and Opportunities for the Clinical Translation of Spatial Transcriptomics Technologies. *Glomerular Diseases*, *4*(1), 49. https://doi.org/10.1159/000538344

Sõber, S., Reiman, M., Kikas, T., Rull, K., Inno, R., Vaas, P., Teesalu, P., Marti, J. M. L., Mattila, P., & Laan, M. (2015). Extensive shift in placental transcriptome profile in preeclampsia and placental origin of adverse pregnancy outcomes. *Scientific Reports 2015 5:1*, *5*(1), 1–17. https://doi.org/10.1038/srep13336

Sonntag, K. C., & Woo, T. U. W. (2018). Laser microdissection and gene expression profiling in the human postmortem brain. *Handbook of Clinical Neurology*, *150*, 263–272. https://doi.org/10.1016/B978-0-444-63639-3.00018-9

Sood, R., Zehnder, J. L., Druzin, M. L., & Brown, P. O. (2006). Gene expression patterns in human placenta. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(14), 5478–5483. https://doi.org/10.1073/PNAS.0508035103

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., & Mesirov, J. P. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(43), 15545–15550. https://doi.org/10.1073/PNAS.0506580102

Sugawara, J., Tazuke, S. I., F-Suen, L., Powell, D. R., Kaper, F., Giaccia, A. J., & Giudice, L. C. (2000). Regulation of insulin-like growth factor-binding protein 1 by hypoxia and 3',5'-cyclic adenosine monophosphate is additive in HepG2 cells. *The Journal of Clinical Endocrinology and Metabolism*, *85*(10), 3821–3827. https://doi.org/10.1210/JCEM.85.10.6866

Sunderland, N., Hennessy, A., & Makris, A. (2011). Animal Models of Pre-eclampsia. *American Journal of Reproductive Immunology*, *65*(6), 533–541. https://doi.org/10.1111/J.1600-0897.2010.00929.X

Szilagyi, A., Gelencser, Z., Romero, R., Xu, Y., Kiraly, P., Demeter, A., Palhalmi, J., Gyorffy, B. A., Juhasz, K., Hupuczi, P., Adrienna, K., Meinhardt, G., Papp, Z., Draghici, S., Erez, O., Tarca, A. L., Knöfler, M., & Gabor, N. (2020a). Placenta-Specific Genes, Their Regulation During Villous Trophoblast Differentiation and Dysregulation in Preterm Preeclampsia. *International Journal of Molecular Sciences 2020, Vol. 21, Page 628*, *21*(2), 628. https://doi.org/10.3390/IJMS21020628

Szilagyi, A., Gelencser, Z., Romero, R., Xu, Y., Kiraly, P., Demeter, A., Palhalmi, J., Gyorffy, B. A., Juhasz, K., Hupuczi, P., Adrienna, K., Meinhardt, G., Papp, Z., Draghici, S., Erez, O., Tarca, A. L., Knöfler, M., & Gabor, N. (2020b). Placenta-Specific Genes, Their Regulation During Villous Trophoblast Differentiation and Dysregulation in Preterm Preeclampsia. *International Journal of Molecular Sciences 2020, Vol. 21, Page 628*, *21*(2), 628. https://doi.org/10.3390/IJMS21020628

Tan, M., Pan, Q., Yu, C., Zhai, X., Gu, J., Tao, L., & Xu, D. (2024). PIGT promotes cell growth, glycolysis, and metastasis in bladder cancer by modulating GLUT1 glycosylation and membrane trafficking. *Journal of Translational Medicine*, *22*(1), 1–16. https://doi.org/10.1186/S12967-023-04805-0/FIGURES/8

Tanbo, T. G., Zucknick, M., & Eskild, A. (2018). Maternal concentrations of human chorionic gonadotrophin in very early IVF pregnancies and duration of pregnancy: a follow-up study. *Reproductive BioMedicine Online*, *37*(2), 208–215. https://doi.org/10.1016/J.RBMO.2018.04.048

Tarca, A. L., Romero, R., & Draghici, S. (2006). Analysis of microarray experiments of gene expression profiling. *American Journal of Obstetrics and Gynecology*, *195*(2), 373. https://doi.org/10.1016/J.AJOG.2006.07.001

Taylor, E. B., & George, E. M. (2022). Animal Models of Preeclampsia: Mechanistic Insights and Promising Therapeutics. *Endocrinology*, *163*(8). https://doi.org/10.1210/ENDOCR/BQAC096

Torchin, H., & Ancel, P. Y. (2016). [Epidemiology and risk factors of preterm birth]. *Journal de Gynecologie, Obstetrique et Biologie de La Reproduction*, *45*(10), 1213–1230. https://doi.org/10.1016/J.JGYN.2016.09.013

Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., Van Baren, M. J., Salzberg, S. L., Wold, B. J., & Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology 2010 28:5*, *28*(5), 511–515. https://doi.org/10.1038/nbt.1621

Tucci, V., Isles, A. R., Kelsey, G., Ferguson-Smith, A. C., Bartolomei, M. S., Benvenisty, N., Bourc'his, D., Charalambous, M., Dulac, C., Feil, R., Glaser, J., Huelsmann, L., John, R. M., McNamara, G. I., Moorwood, K., Muscatelli, F., Sasaki, H., Strassmann, B. I., Vincenz, C., & Wilkins, J. (2019). Genomic Imprinting and Physiological Processes in Mammals. *Cell*, *176*(5), 952–965. https://doi.org/10.1016/J.CELL.2019.01.043

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I. M., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A. K., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., … Pontén, F. (2015). Tissue-based map of the human proteome. *Science*, *347*(6220). https://doi.org/10.1126/SCIENCE.1260419/SUPPL_FILE/1260419_UHLEN.SM.PDF

Umesawa, M., & Kobashi, G. (2016). Epidemiology of hypertensive disorders in pregnancy: prevalence, risk factors, predictors and prognosis. *Hypertension Research 2017 40:3*, *40*(3), 213–220. https://doi.org/10.1038/hr.2016.126

Van Oppenraaij, R. H. F., Jauniaux, E., Christiansen, O. B., Horcajadas, J. A., Farquharson, R. G., & Exalto, N. (2009). Predicting adverse obstetric outcome after early pregnancy events and complications: a review. *Human Reproduction Update*, *15*(4), 409–421. https://doi.org/10.1093/HUMUPD/DMP009

Van Rossum, G. (n.d.). *Centrum voor Wiskunde en Informatica Python tutorial*.

Vento-Tormo, R., Efremova, M., Botting, R. A., Turco, M. Y., Vento-Tormo, M., Meyer, K. B., Park, J. E., Stephenson, E., Polański, K., Goncalves, A., Gardner, L., Holmqvist, S., Henriksson, J., Zou, A., Sharkey, A. M., Millar, B., Innes, B., Wood, L., Wilbrey-Clark, A., … Teichmann, S. A. (2018). Single-cell reconstruction of the early maternal–fetal interface in humans. *Nature 2018 563:7731*, *563*(7731), 347–353. https://doi.org/10.1038/s41586-018-0698-6

Wang, L., Wang, S., & Li, W. (2012). RSeQC: quality control of RNA-seq experiments. *Bioinformatics (Oxford, England)*, *28*(16), 2184–2185. https://doi.org/10.1093/BIOINFORMATICS/BTS356

Wang, X., Miller, D. C., Harman, R., Antczak, D. F., & Clark, A. G. (2013). Paternally expressed genes predominate in the placenta. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(26), 10705–10710. https://doi.org/10.1073/PNAS.1308998110

Weissgerber, T. L., & Mudd, L. M. (2015). Preeclampsia and Diabetes. *Current Diabetes Reports*, *15*(3), 1–10. https://doi.org/10.1007/S11892-015-0579-4/TABLES/1

Xu, P., Ma, Y., Wu, H., & Wang, Y. L. (2021). Placenta-Derived MicroRNAs in the Pathophysiology of Human Pregnancy. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/FCELL.2021.646326

Yong, H. E. J., & Chan, S. Y. (2020). Current approaches and developments in transcript profiling of the human placenta. *Human Reproduction Update*, *26*(6), 799. https://doi.org/10.1093/HUMUPD/DMAA028

Youssef, A., Vermeulen, N., Lashley, E. E. L. O., Goddijn, M., & van der Hoorn, M. L. P. (2019). Comparison and appraisal of (inter)national recurrent pregnancy loss guidelines. *Reproductive BioMedicine Online*, *39*(3), 497–503. https://doi.org/10.1016/J.RBMO.2019.04.008

Zhao, S., Zhang, Y., Gamini, R., Zhang, B., & Von Schack, D. (2018). Evaluation of two main RNA-seq approaches for gene quantification in clinical RNA sequencing: polyA+ selection versus rRNA depletion. *Scientific Reports 2018 8:1*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-23226-4

Zou, Z., Forbes, K., Harris, L. K., & Heazell, A. E. P. (2021). The potential role of the E SRRG pathway in placental dysfunction. *Reproduction*, *161*(3), R45–R60. https://doi.org/10.1530/REP-20-0272

ACKNOWLEDGMENTS

I want to express my deepest gratitude to my supervisor, Maris Laan, for her guidance, support, and invaluable insights throughout my career in science. Her expertise and encouragement have been instrumental in shaping this work.

I am also indebted to my initial supervisor, Siim Sõber, for laying the foundation of my research and providing me with the necessary tools and knowledge to embark on this academic journey.

I am thankful to my current and past lab mates, especially Rain, Triin, Diana, Anna Maria, Mart, Kristiina, Laura, Eve and Eveliis, for their camaraderie, intellectual exchanges, and support. Their presence has made the research environment both stimulating and enjoyable.

Additional thanks go to my team lead at Eesti Energia, Erko Varblane, for always being understanding and supportive of my studies and never objecting to me taking study vacations.

I want to extend my appreciation to Professor Maido Remm for reviewing this thesis and offering constructive feedback that has significantly improved the quality of this work.

Finally, I thank my family for their constant love, understanding, and belief in my abilities. I sincerely apologize for not being able to spend as much time with them as I would have liked due to balancing a day job, doctoral school, and family life at the same time. Their unwavering support has been my source of strength throughout this journey.

PUBLICATIONS

CURRICULUM VITAE

Name:	Mario Reiman
Date of birth:	February 20, 1991
E-mail:	mario.reiman@ut.ee
Telephone:	+372 5810 5966

Education:

2015–2024	PhD student in Gene Technology, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu, Estonia
2013–2014	Master's degree in Gene Technology, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu, Estonia
2010–2013	Bachelor's degree in Gene Technology, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu, Estonia

Professional employment:

2024–…	Enefit AS, Senior Data Analyst
2019–2023	Eesti Energia AS, Senior Data Analyst
2019	Eesti Energia AS, Data Analyst
2019	University of Tartu, Faculty of Medicine, Institute of Biomedicine and Translational Medicine, Assistant in Human Genetics
2016–2017	University of Tartu, Faculty of Medicine, Institute of Biomedicine and Translational Medicine, Assistant in Human Genetics

Publications:

Siim Sõber, **Mario Reiman**, Triin Kikas, Kristiina Rull, Rain Inno, Pille Vaas, Pille Teesalu, Jesus M.Lopez Marti, Pirkko Mattila, and Maris Laan. 2015. "Extensive Shift in Placental Transcriptome Profile in Preeclampsia and Placental Origin of Adverse Pregnancy Outcomes." Scientific Reports 2015 5:1 5 (1): 1–17. https://doi.org/10.1038/srep13336.

Siim Sõber, Kristiina Rull, **Mario Reiman**, Piret Ilisson, Pirkko Mattila, and Maris Laan. 2016. "RNA Sequencing of Chorionic Villi from Recurrent Pregnancy Loss Patients Reveals Impaired Function of Basic Nuclear and Cellular Machinery." Scientific Reports 2016 6:1 6 (1): 1–14. https://doi.org/10.1038/srep38439.

Mario Reiman, Maris Laan, Kristiina Rull, and Siim Sõber. 2017. "Effects of RNA Integrity on Transcript Quantification by Total RNA Sequencing of Clinically Collected Human Placental Samples." The FASEB Journal 31 (8): 3298–3308. https://doi.org/10.1096/FJ.201601031RR.

Diana Pilvar, **Mario Reiman**, Arno Pilvar, and Maris Laan. 2019. "Parent-of-Origin-Specific Allelic Expression in the Human Placenta Is Limited to Established Imprinted Loci and It Is Stably Maintained across Pregnancy." Clinical Epigenetics 11 (1): 1–14. https://doi.org/10.1186/S13148-019-0692-3.

Triin Kikas, Anna Maria Punab, Laura Kasak, Olev Poolamets, Vladimir Vihljajev, Kristjan Pomm, **Mario Reiman**, Stanislav Tjagur, Paul Korrovits, Margus Punab, and Maris Laan. 2023. "Microdeletions and Microduplications Linked to Severe Congenital Disorders in Infertile Men." Scientific Reports 13 (1). https://doi.org/10.1038/S41598-023-27750-W.

Awards and stipends:

2018 Biomedicine and Biotechnology doctoral school scholarship to attend "European Human Genetics Conference", Milan, Italy and "Eurotoxemia 2018: the European meeting on preeclampsia" Paris, France

2018 COST travel reimbursement for Epigenetic Chemical Biology ECI Workshop and Core Group Meeting, Paris, France

2017 Dora Plus Scholarship for short-term study mobility: poster presentation at the EMBO | EMBL Symposium: From Single- to Multiomics: "Applications and Challenges in Data Integration" attendance, Heidelberg, Germany

2016 Kristjan Jaak Fellowship to attend EMBL-EBI course "Introduction to Omics data integration", Hinxton, United Kingdom

Supervised dissertations:

2019 Käbi-Riin Ojassoo, BSc in Gene Technology, supervisors: Triin Kikas, Mario Reiman, Mihkel Vaher; Alternative splicing in normal and pre-eclamptic pregnancies; Faculty of Science and Technology, University of Tartu

2019 Eveliis Koppel, MSc in Biomedicine, supervisors: Mario Reiman, Maris Laan; Dynamics of gene expression in placenta during pregnancy and connection to pregnancy complications; Faculty of Science and Technology, University of Tartu

ELULOOKIRJELDUS

Nimi: Mario Reiman
Sünniaeg: 20.02.1991
E-post: mario.reiman@ut.ee
Telefon: +372 58105966

Hariduskäik:

2015–2024	Doktorant geenitehnoloogia õppekaval, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2013–2014	Loodusteaduste magistrikraadkraad (MSc) geenitehnoloogia õppekaval, Molekulaar ja Rakubioloogia Instituut, Tartu Ülikool
2010–2013	Loodusteaduste bakalaureusekraad (BSc) geenitehnoloogia õppekaval, Molekulaar ja Rakubioloogia Instituut, Tartu Ülikool

Teenistuskäik:

2024–…	Enefit AS, vanem andmeanalüütik
2019–2023	Eesti Energia AS, vanem andmeanalüütik
2019	Eesti Energia AS, andmeanalüütik
2019	Tartu Ülikool, Meditsiiniteaduste valdkond, Bio ja Siirdemeditsiini Instituut, inimesegeneetika assistent
2016–2017	Tartu Ülikool, Meditsiiniteaduste valdkond, Bio ja Siirdemeditsiini Instituut, inimesegeneetika assistent

Teaduspublikatsioonid:

Siim Sõber, Mario Reiman, Triin Kikas, Kristiina Rull, Rain Inno, Pille Vaas, Pille Teesalu, Jesus M.Lopez Marti, Pirkko Mattila, ja Maris Laan. 2015. “Extensive Shift in Placental Transcriptome Profile in Preeclampsia and Placental Origin of Adverse Pregnancy Outcomes.” Scientific Reports 2015 5:1 5 (1): 1–17. https://doi.org/10.1038/srep13336.

Siim Sõber, Kristiina Rull, Mario Reiman, Piret Ilisson, Pirkko Mattila, ja Maris Laan. 2016. “RNA Sequencing of Chorionic Villi from Recurrent Pregnancy Loss Patients Reveals Impaired Function of Basic Nuclear and Cellular Machinery.” Scientific Reports 2016 6:1 6 (1): 1–14. https://doi.org/10.1038/srep38439.

Mario Reiman, Maris Laan, Kristiina Rull, ja Siim Sõber. 2017. “Effects of RNA Integrity on Transcript Quantification by Total RNA Sequencing of Clinically Collected Human Placental Samples.” The FASEB Journal 31 (8): 3298–3308. https://doi.org/10.1096/FJ.201601031RR.

Diana Pilvar, **Mario Reiman**, Arno Pilvar, and Maris Laan. 2019. "Parent-of-Origin-Specific Allelic Expression in the Human Placenta Is Limited to Established Imprinted Loci and It Is Stably Maintained across Pregnancy." Clinical Epigenetics 11 (1): 1–14. https://doi.org/10.1186/S13148-019-0692-3.

Triin Kikas, Anna Maria Punab, Laura Kasak, Olev Poolamets, Vladimir Vihljajev, Kristjan Pomm, **Mario Reiman**, Stanislav Tjagur, Paul Korrovits, Margus Punab, and Maris Laan. 2023. "Microdeletions and Microduplications Linked to Severe Congenital Disorders in Infertile Men." Scientific Reports 13 (1). https://doi.org/10.1038/S41598-023-27750-W.

Awards and stipends:

2018 Biomedicine and Biotechnology doctoral school scholarship to attend "European Human Genetics Conference", Milan, Italy and "Eurotoxemia 2018: the European meeting on preeclampsia" Paris, France

2018 COST travel reimbursement for Epigenetic Chemical Biology ECI Workshop and Core Group Meeting, Paris, France

2017 Dora Plus Scholarship for short-term study mobility: poster presentation at the EMBO | EMBL Symposium: From Single- to Multiomics: "Applications and Challenges in Data Integration" attendance, Heidelberg, Germany

2016 Kristjan Jaak Fellowship to attend EMBL-EBI course "Introduction to Omics data integration", Hinxton, United Kingdom

Supervised dissertations:

2019 Käbi-Riin Ojassoo, BSc in Gene Technology, supervisors: Triin Kikas, Mario Reiman, Mihkel Vaher; Alternative splicing in normal and pre-eclamptic pregnancies; Faculty of Science and Technology, University of Tartu

2019 Eveliis Koppel, MSc in Biomedicine, supervisors: Mario Reiman, Maris Laan; Dynamics of gene expression in placenta during pregnancy and connection to pregnancy complications; Faculty of Science and Technology, University of Tartu

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous grassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan**. Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu**. Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.

42. **Veljo Kisand**. Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa**. Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa**. Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik**. Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo**. Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo**. Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots**. Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero**. Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees**. Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks**. Cholecystokinin (CCK) – induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and serotonin. Tartu, 1999, 123 p.
52. **Ebe Sild**. Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva**. Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna**. Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro**. Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane**. Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm**. Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg**. Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild**. The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.
60. **Niilo Kaldalu**. Studies of the TOL plasmid transcription factor XylS. Tartu, 2000, 88 p.
61. **Dina Lepik**. Modulation of viral DNA replication by tumor suppressor protein p53. Tartu, 2000, 106 p.
62. **Kai Vellak**. Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu, 2000, 122 p.

63. **Jonne Kotta**. Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu, 2000, 160 p.
64. **Georg Martin**. Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000, 139 p.
65. **Silvia Sepp**. Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira**. On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000, 96 p.
67. **Priit Zingel**. The role of planktonic ciliates in lake ecosystems. Tartu, 2001, 111 p.
68. **Tiit Teder**. Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu, 2001, 122 p.
69. **Hannes Kollist**. Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu, 2001, 80 p.
70. **Reet Marits**. Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu, 2001, 112 p.
71. **Vallo Tilgar**. Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002, 126 p.
72. **Rita Hõrak**. Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002, 108 p.
73. **Liina Eek-Piirsoo**. The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002, 74 p.
74. **Krõõt Aasamaa**. Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002, 110 p.
75. **Nele Ingerpuu**. Bryophyte diversity and vascular plants. Tartu, 2002, 112 p.
76. **Neeme Tõnisson**. Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002, 124 p.
77. **Margus Pensa**. Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003, 110 p.
78. **Asko Lõhmus**. Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003, 168 p.
79. **Viljar Jaks**. p53 – a switch in cellular circuit. Tartu, 2003, 160 p.
80. **Jaana Männik**. Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003, 140 p.
81. **Marek Sammul**. Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003, 159 p
82. **Ivar Ilves**. Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003, 89 p.
83. **Andres Männik**. Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003, 109 p.

84. **Ivika Ostonen**. Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003, 158 p.
85. **Gudrun Veldre**. Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003, 199 p.
86. **Ülo Väli.** The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004, 159 p.
87. **Aare Abroi**. The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004, 135 p.
88. **Tiina Kahre**. Cystic fibrosis in Estonia. Tartu, 2004, 116 p.
89. **Helen Orav-Kotta**. Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004, 117 p.
90. **Maarja Öpik**. Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004, 175 p.
91. **Kadri Tali**. Species structure of *Neotinea ustulata*. Tartu, 2004, 109 p.
92. **Kristiina Tambets**. Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004, 163 p.
93. **Arvi Jõers**. Regulation of p53-dependent transcription. Tartu, 2004, 103 p.
94. **Lilian Kadaja**. Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004, 103 p.
95. **Jaak Truu**. Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004, 128 p.
96. **Maire Peters**. Natural horizontal transfer of the *pheBA* operon. Tartu, 2004, 105 p.
97. **Ülo Maiväli**. Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004, 130 p.
98. **Merit Otsus**. Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004, 103 p.
99. **Mikk Heidemaa**. Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004, 167 p.
100. **Ilmar Tõnno**. The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004, 111 p.
101. **Lauri Saks**. Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004, 144 p.
102. **Siiri Rootsi**. Human Y-chromosomal variation in European populations. Tartu, 2004, 142 p.
103. **Eve Vedler**. Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.
104. **Andres Tover**. Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 126 p.
105. **Helen Udras**. Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005, 100 p.

106. **Ave Suija**. Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005, 162 p.
107. **Piret Lõhmus**. Forest lichens and their substrata in Estonia. Tartu, 2005, 162 p.
108. **Inga Lips**. Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005, 156 p.
109. **Krista Kaasik**. Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005, 121 p.
110. **Juhan Javoiš**. The effects of experience on host acceptance in ovipositing moths. Tartu, 2005, 112 p.
111. **Tiina Sedman**. Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005, 103 p.
112. **Ruth Aguraiuja**. Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005, 112 p.
113. **Riho Teras**. Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 106 p.
114. **Mait Metspalu**. Through the course of prehistory in India: tracing the mtDNA trail. Tartu, 2005, 138 p.
115. **Elin Lõhmussaar**. The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006, 124 p.
116. **Priit Kupper**. Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006, 126 p.
117. **Heili Ilves**. Stress-induced transposition of Tn*4652* in *Pseudomonas Putida.* Tartu, 2006, 120 p.
118. **Silja Kuusk**. Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006, 126 p.
119. **Kersti Püssa**. Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006, 90 p.
120. **Lea Tummeleht**. Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006, 94 p.
121. **Toomas Esperk**. Larval instar as a key element of insect growth schedules. Tartu, 2006, 186 p.
122. **Harri Valdmann**. Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.
123. **Priit Jõers**. Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.
124. **Kersti Lilleväli**. Gata3 and Gata2 in inner ear development. Tartu, 2007, 123 p.
125. **Kai Rünk**. Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007, 143 p.

126. **Aveliina Helm**. Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007, 89 p.
127. **Leho Tedersoo**. Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007, 233 p.
128. **Marko Mägi**. The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007, 135 p.
129. **Valeria Lulla**. Replication strategies and applications of Semliki Forest virus. Tartu, 2007, 109 p.
130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007, 79 p.
131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007, 171 p.
132. **Tatjana Krama**. Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007, 112 p.
133. **Signe Saumaa**. The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida*. Tartu, 2007, 172 p.
134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007, 96 p.
135. **Priit Kilgas**. Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007, 129 p.
136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007, 95 p.
137. **Kärt Padari**. Protein transduction mechanisms of transportans. Tartu, 2008, 128 p.
138. **Siiri-Lii Sandre**. Selective forces on larval colouration in a moth. Tartu, 2008, 125 p.
139. **Ülle Jõgar**. Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008, 99 p.
140. **Lauri Laanisto**. Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008, 133 p.
141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008, 105 p.
142. **Birgot Paavel**. Bio-optical properties of turbid lakes. Tartu, 2008, 175 p.
143. **Kaire Torn**. Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.
144. **Vladimir Vimberg**. Peptide mediated macrolide resistance. Tartu, 2008, 190 p.
145. **Daima Örd**. Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.
146. **Lauri Saag**. Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.

147. **Ulvi Karu**. Antioxidant protection, carotenoids and coccidians in greenfinches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm**. Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks**. Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu**. Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš**. Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko**. Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast**. Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats**. Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.
156. **Radi Tegova**. The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida*. Tartu, 2009, 124 p.
157. **Tsipe Aavik**. Plant species richness, composition and functional trait pattern in agricultural landscapes – the role of land use intensity and landscape structure. Tartu, 2009, 112 p.
158. **Kaja Kiiver**. Semliki forest virus based vectors and cell lines for studying the replication and interactions of alphaviruses and hepaciviruses. Tartu, 2009, 104 p.
159. **Meelis Kadaja**. Papillomavirus Replication Machinery Induces Genomic Instability in its Host Cell. Tartu, 2009, 126 p.
160. **Pille Hallast**. Human and chimpanzee Luteinizing hormone/Chorionic Gonadotropin beta (*LHB/CGB*) gene clusters: diversity and divergence of young duplicated genes. Tartu, 2009, 168 p.
161. **Ain Vellak**. Spatial and temporal aspects of plant species conservation. Tartu, 2009, 86 p.
162. **Triinu Remmel**. Body size evolution in insects with different colouration strategies: the role of predation risk. Tartu, 2009, 168 p.
163. **Jaana Salujõe**. Zooplankton as the indicator of ecological quality and fish predation in lake ecosystems. Tartu, 2009, 129 p.
164. **Ele Vahtmäe**. Mapping benthic habitat with remote sensing in optically complex coastal environments. Tartu, 2009, 109 p.
165. **Liisa Metsamaa**. Model-based assessment to improve the use of remote sensing in recognition and quantitative mapping of cyanobacteria. Tartu, 2009, 114 p.

166. **Pille Säälik**. The role of endocytosis in the protein transduction by cell-penetrating peptides. Tartu, 2009, 155 p.
167. **Lauri Peil**. Ribosome assembly factors in *Escherichia coli*. Tartu, 2009, 147 p.
168. **Lea Hallik**. Generality and specificity in light harvesting, carbon gain capacity and shade tolerance among plant functional groups. Tartu, 2009, 99 p.
169. **Mariliis Tark**. Mutagenic potential of DNA damage repair and tolerance mechanisms under starvation stress. Tartu, 2009, 191 p.
170. **Riinu Rannap**. Impacts of habitat loss and restoration on amphibian populations. Tartu, 2009, 117 p.
171. **Maarja Adojaan**. Molecular variation of HIV-1 and the use of this knowledge in vaccine development. Tartu, 2009, 95 p.
172. **Signe Altmäe**. Genomics and transcriptomics of human induced ovarian folliculogenesis. Tartu, 2010, 179 p.
173. **Triin Suvi**. Mycorrhizal fungi of native and introduced trees in the Seychelles Islands. Tartu, 2010, 107 p.
174. **Velda Lauringson**. Role of suspension feeding in a brackish-water coastal sea. Tartu, 2010, 123 p.
175. **Eero Talts**. Photosynthetic cyclic electron transport – measurement and variably proton-coupled mechanism. Tartu, 2010, 121 p.
176. **Mari Nelis**. Genetic structure of the Estonian population and genetic distance from other populations of European descent. Tartu, 2010, 97 p.
177. **Kaarel Krjutškov**. Arrayed Primer Extension-2 as a multiplex PCR-based method for nucleic acid variation analysis: method and applications. Tartu, 2010, 129 p.
178. **Egle Köster**. Morphological and genetical variation within species complexes: *Anthyllis vulneraria* s. l. and *Alchemilla vulgaris* (coll.). Tartu, 2010, 101 p.
179. **Erki Õunap**. Systematic studies on the subfamily Sterrhinae (Lepidoptera: Geometridae). Tartu, 2010, 111 p.
180. **Merike Jõesaar**. Diversity of key catabolic genes at degradation of phenol and *p*-cresol in pseudomonads. Tartu, 2010, 125 p.
181. **Kristjan Herkül**. Effects of physical disturbance and habitat-modifying species on sediment properties and benthic communities in the northern Baltic Sea. Tartu, 2010, 123 p.
182. **Arto Pulk**. Studies on bacterial ribosomes by chemical modification approaches. Tartu, 2010, 161 p.
183. **Maria Põllupüü**. Ecological relations of cladocerans in a brackish-water ecosystem. Tartu, 2010, 126 p.
184. **Toomas Silla**. Study of the segregation mechanism of the Bovine Papillomavirus Type 1. Tartu, 2010, 188 p.
185. **Gyaneshwer Chaubey**. The demographic history of India: A perspective based on genetic evidence. Tartu, 2010, 184 p.

186. **Katrin Kepp**. Genes involved in cardiovascular traits: detection of genetic variation in Estonian and Czech populations. Tartu, 2010, 164 p.
187. **Virve Sõber**. The role of biotic interactions in plant reproductive performance. Tartu, 2010, 92 p.
188. **Kersti Kangro**. The response of phytoplankton community to the changes in nutrient loading. Tartu, 2010, 144 p.
189. **Joachim M. Gerhold**. Replication and Recombination of mitochondrial DNA in Yeast. Tartu, 2010, 120 p.
190. **Helen Tammert**. Ecological role of physiological and phylogenetic diversity in aquatic bacterial communities. Tartu, 2010, 140 p.
191. **Elle Rajandu**. Factors determining plant and lichen species diversity and composition in Estonian *Calamagrostis* and *Hepatica* site type forests. Tartu, 2010, 123 p.
192. **Paula Ann Kivistik**. ColR-ColS signalling system and transposition of Tn*4652* in the adaptation of *Pseudomonas putida*. Tartu, 2010, 118 p.
193. **Siim Sõber**. Blood pressure genetics: from candidate genes to genome-wide association studies. Tartu, 2011, 120 p.
194. **Kalle Kipper**. Studies on the role of helix 69 of 23S rRNA in the factor-dependent stages of translation initiation, elongation, and termination. Tartu, 2011, 178 p.
195. **Triinu Siibak**. Effect of antibiotics on ribosome assembly is indirect. Tartu, 2011, 134 p.
196. **Tambet Tõnissoo**. Identification and molecular analysis of the role of guanine nucleotide exchange factor RIC-8 in mouse development and neural function. Tartu, 2011, 110 p.
197. **Helin Räägel**. Multiple faces of cell-penetrating peptides – their intracellular trafficking, stability and endosomal escape during protein transduction. Tartu, 2011, 161 p.
198. **Andres Jaanus**. Phytoplankton in Estonian coastal waters – variability, trends and response to environmental pressures. Tartu, 2011, 157 p.
199. **Tiit Nikopensius**. Genetic predisposition to nonsyndromic orofacial clefts. Tartu, 2011, 152 p.
200. **Signe Värv**. Studies on the mechanisms of RNA polymerase II-dependent transcription elongation. Tartu, 2011, 108 p.
201. **Kristjan Välk**. Gene expression profiling and genome-wide association studies of non-small cell lung cancer. Tartu, 2011, 98 p.
202. **Arno Põllumäe**. Spatio-temporal patterns of native and invasive zooplankton species under changing climate and eutrophication conditions. Tartu, 2011, 153 p.
203. **Egle Tammeleht**. Brown bear (*Ursus arctos*) population structure, demographic processes and variations in diet in northern Eurasia. Tartu, 2011, 143 p.
205. **Teele Jairus**. Species composition and host preference among ectomycorrhizal fungi in Australian and African ecosystems. Tartu, 2011, 106 p.

206. **Kessy Abarenkov**. PlutoF – cloud database and computing services supporting biological research. Tartu, 2011, 125 p.
207. **Marina Grigorova**. Fine-scale genetic variation of follicle-stimulating hormone beta-subunit coding gene (*FSHB*) and its association with reproductive health. Tartu, 2011, 184 p.
208. **Anu Tiitsaar**. The effects of predation risk and habitat history on butterfly communities. Tartu, 2011, 97 p.
209. **Elin Sild**. Oxidative defences in immunoecological context: validation and application of assays for nitric oxide production and oxidative burst in a wild passerine. Tartu, 2011, 105 p.
210. **Irja Saar**. The taxonomy and phylogeny of the genera *Cystoderma* and *Cystodermella* (Agaricales, Fungi). Tartu, 2012, 167 p.
211. **Pauli Saag**. Natural variation in plumage bacterial assemblages in two wild breeding passerines. Tartu, 2012, 113 p.
212. **Aleksei Lulla**. Alphaviral nonstructural protease and its polyprotein substrate: arrangements for the perfect marriage. Tartu, 2012, 143 p.
213. **Mari Järve**. Different genetic perspectives on human history in Europe and the Caucasus: the stories told by uniparental and autosomal markers. Tartu, 2012, 119 p.
214. **Ott Scheler**. The application of tmRNA as a marker molecule in bacterial diagnostics using microarray and biosensor technology. Tartu, 2012, 93 p.
215. **Anna Balikova**. Studies on the functions of tumor-associated mucin-like leukosialin (CD43) in human cancer cells. Tartu, 2012, 129 p.
216. **Triinu Kõressaar**. Improvement of PCR primer design for detection of prokaryotic species. Tartu, 2012, 83 p.
217. **Tuul Sepp**. Hematological health state indices of greenfinches: sources of individual variation and responses to immune system manipulation. Tartu, 2012, 117 p.
218. **Rya Ero**. Modifier view of the bacterial ribosome. Tartu, 2012, 146 p.
219. **Mohammad Bahram**. Biogeography of ectomycorrhizal fungi across different spatial scales. Tartu, 2012, 165 p.
220. **Annely Lorents**. Overcoming the plasma membrane barrier: uptake of amphipathic cell-penetrating peptides induces influx of calcium ions and downstream responses. Tartu, 2012, 113 p.
221. **Katrin Männik**. Exploring the genomics of cognitive impairment: whole-genome SNP genotyping experience in Estonian patients and general population. Tartu, 2012, 171 p.
222. **Marko Prous**. Taxonomy and phylogeny of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae). Tartu, 2012, 192 p.
223. **Triinu Visnapuu**. Levansucrases encoded in the genome of *Pseudomonas syringae* pv. tomato DC3000: heterologous expression, biochemical characterization, mutational analysis and spectrum of polymerization products. Tartu, 2012, 160 p.
224. **Nele Tamberg**. Studies on Semliki Forest virus replication and pathogenesis. Tartu, 2012, 109 p.

225. **Tõnu Esko**. Novel applications of SNP array data in the analysis of the genetic structure of Europeans and in genetic association studies. Tartu, 2012, 149 p.
226. **Timo Arula**. Ecology of early life-history stages of herring *Clupea harengus membras* in the northeastern Baltic Sea. Tartu, 2012, 143 p.
227. **Inga Hiiesalu**. Belowground plant diversity and coexistence patterns in grassland ecosystems. Tartu, 2012, 130 p.
228. **Kadri Koorem**. The influence of abiotic and biotic factors on small-scale plant community patterns and regeneration in boreonemoral forest. Tartu, 2012, 114 p.
229. **Liis Andresen**. Regulation of virulence in plant-pathogenic pectobacteria. Tartu, 2012, 122 p.
230. **Kaupo Kohv**. The direct and indirect effects of management on boreal forest structure and field layer vegetation. Tartu, 2012, 124 p.
231. **Mart Jüssi**. Living on an edge: landlocked seals in changing climate. Tartu, 2012, 114 p.
232. **Riina Klais**. Phytoplankton trends in the Baltic Sea. Tartu, 2012, 136 p.
233. **Rauno Veeroja**. Effects of winter weather, population density and timing of reproduction on life-history traits and population dynamics of moose (*Alces alces*) in Estonia. Tartu, 2012, 92 p.
234. **Marju Keis**. Brown bear (*Ursus arctos*) phylogeography in northern Eurasia. Tartu, 2013, 142 p.
235. **Sergei Põlme**. Biogeography and ecology of *alnus*- associated ectomycorrhizal fungi – from regional to global scale. Tartu, 2013, 90 p.
236. **Liis Uusküla**. Placental gene expression in normal and complicated pregnancy. Tartu, 2013, 173 p.
237. **Marko Lõoke**. Studies on DNA replication initiation in *Saccharomyces cerevisiae*. Tartu, 2013, 112 p.
238. **Anne Aan**. Light- and nitrogen-use and biomass allocation along productivity gradients in multilayer plant communities. Tartu, 2013, 127 p.
239. **Heidi Tamm**. Comprehending phylogenetic diversity – case studies in three groups of ascomycetes. Tartu, 2013, 136 p.
240. **Liina Kangur**. High-Pressure Spectroscopy Study of Chromophore-Binding Hydrogen Bonds in Light-Harvesting Complexes of Photosynthetic Bacteria. Tartu, 2013, 150 p.
241. **Margus Leppik**. Substrate specificity of the multisite specific pseudouridine synthase RluD. Tartu, 2013, 111 p.
242. **Lauris Kaplinski**. The application of oligonucleotide hybridization model for PCR and microarray optimization. Tartu, 2013, 103 p.
243. **Merli Pärnoja**. Patterns of macrophyte distribution and productivity in coastal ecosystems: effect of abiotic and biotic forcing. Tartu, 2013, 155 p.
244. **Tõnu Margus**. Distribution and phylogeny of the bacterial translational GTPases and the Mqsr/YgiT regulatory system. Tartu, 2013, 126 p.
245. **Pille Mänd**. Light use capacity and carbon and nitrogen budget of plants: remote assessment and physiological determinants. Tartu, 2013, 128 p.

246. **Mario Plaas**. Animal model of Wolfram Syndrome in mice: behavioural, biochemical and psychopharmacological characterization. Tartu, 2013, 144 p.
247. **Georgi Hudjašov**. Maps of mitochondrial DNA, Y-chromosome and tyrosinase variation in Eurasian and Oceanian populations. Tartu, 2013, 115 p.
248. **Mari Lepik**. Plasticity to light in herbaceous plants and its importance for community structure and diversity. Tartu, 2013, 102 p.
249. **Ede Leppik**. Diversity of lichens in semi-natural habitats of Estonia. Tartu, 2013, 151 p.
250. **Ülle Saks**. Arbuscular mycorrhizal fungal diversity patterns in boreo-nemoral forest ecosystems. Tartu, 2013, 151 p.
251. **Eneli Oitmaa**. Development of arrayed primer extension microarray assays for molecular diagnostic applications. Tartu, 2013, 147 p.
252. **Jekaterina Jutkina**. The horizontal gene pool for aromatics degradation: bacterial catabolic plasmids of the Baltic Sea aquatic system. Tartu, 2013, 121 p.
253. **Helen Vellau**. Reaction norms for size and age at maturity in insects: rules and exceptions. Tartu, 2014, 132 p.
254. **Randel Kreitsberg**. Using biomarkers in assessment of environmental contamination in fish – new perspectives. Tartu, 2014, 107 p.
255. **Krista Takkis**. Changes in plant species richness and population performance in response to habitat loss and fragmentation.Tartu, 2014, 141 p.
256. **Liina Nagirnaja**. Global and fine-scale genetic determinants of recurrent pregnancy loss. Tartu, 2014, 211 p.
257. **Triin Triisberg**. Factors influencing the re-vegetation of abandoned extracted peatlands in Estonia. Tartu, 2014, 133 p.
258. **Villu Soon**. A phylogenetic revision of the *Chrysis ignita* species group (Hymenoptera: Chrysididae) with emphasis on the northern European fauna. Tartu, 2014, 211 p.
259. **Andrei Nikonov**. RNA-Dependent RNA Polymerase Activity as a Basis for the Detection of Positive-Strand RNA Viruses by Vertebrate Host Cells. Tartu, 2014, 207 p.
260. **Eele Õunapuu-Pikas**. Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences. Tartu, 2014, 135 p.
261. **Marju Männiste**. Physiological ecology of greenfinches: information content of feathers in relation to immune function and behavior. Tartu, 2014, 121 p.
262. **Katre Kets**. Effects of elevated concentrations of CO_2 and O_3 on leaf photosynthetic parameters in *Populus tremuloides*: diurnal, seasonal and inter-annual patterns. Tartu, 2014, 115 p.
263. **Külli Lokko**. Seasonal and spatial variability of zoopsammon communities in relation to environmental parameters. Tartu, 2014, 129 p.
264. **Olga Žilina**. Chromosomal microarray analysis as diagnostic tool: Estonian experience. Tartu, 2014, 152 p.

265. **Kertu Lõhmus**. Colonisation ecology of forest-dwelling vascular plants and the conservation value of rural manor parks. Tartu, 2014, 111 p.
266. **Anu Aun**. Mitochondria as integral modulators of cellular signaling. Tartu, 2014, 167 p.
267. **Chandana Basu Mallick**. Genetics of adaptive traits and gender-specific demographic processes in South Asian populations. Tartu, 2014, 160 p.
268. **Riin Tamme**. The relationship between small-scale environmental heterogeneity and plant species diversity. Tartu, 2014, 130 p.
269. **Liina Remm**. Impacts of forest drainage on biodiversity and habitat quality: implications for sustainable management and conservation. Tartu, 2015, 126 p.
270. **Tiina Talve**. Genetic diversity and taxonomy within the genus *Rhinanthus*. Tartu, 2015, 106 p.
271. **Mehis Rohtla**. Otolith sclerochronological studies on migrations, spawning habitat preferences and age of freshwater fishes inhabiting the Baltic Sea. Tartu, 2015, 137 p.
272. **Alexey Reshchikov**. The world fauna of the genus *Lathrolestes* (Hymenoptera, Ichneumonidae). Tartu, 2015, 247 p.
273. **Martin Pook**. Studies on artificial and extracellular matrix protein-rich surfaces as regulators of cell growth and differentiation. Tartu, 2015, 142 p.
274. **Mai Kukumägi**. Factors affecting soil respiration and its components in silver birch and Norway spruce stands. Tartu, 2015, 155 p.
275. **Helen Karu**. Development of ecosystems under human activity in the North-East Estonian industrial region: forests on post-mining sites and bogs. Tartu, 2015, 152 p.
276. **Hedi Peterson**. Exploiting high-throughput data for establishing relationships between genes. Tartu, 2015, 186 p.
277. **Priit Adler**. Analysis and visualisation of large scale microarray data, Tartu, 2015, 126 p.
278. **Aigar Niglas**. Effects of environmental factors on gas exchange in deciduous trees: focus on photosynthetic water-use efficiency. Tartu, 2015, 152 p.
279. **Silja Laht**. Classification and identification of conopeptides using profile hidden Markov models and position-specific scoring matrices. Tartu, 2015, 100 p.
280. **Martin Kesler**. Biological characteristics and restoration of Atlantic salmon *Salmo salar* populations in the Rivers of Northern Estonia. Tartu, 2015, 97 p.
281. **Pratyush Kumar Das**. Biochemical perspective on alphaviral nonstructural protein 2: a tale from multiple domains to enzymatic profiling. Tartu, 2015, 205 p
282. **Priit Palta**. Computational methods for DNA copy number detection. Tartu, 2015, 130 p.
283. **Julia Sidorenko**. Combating DNA damage and maintenance of genome integrity in pseudomonads. Tartu, 2015, 174 p.

284. **Anastasiia Kovtun-Kante**. Charophytes of Estonian inland and coastal waters: distribution and environmental preferences. Tartu, 2015, 97 p.
285. **Ly Lindman**. The ecology of protected butterfly species in Estonia. Tartu, 2015, 171 p.
286. **Jaanis Lodjak**. Association of Insulin-like Growth Factor I and Corticosterone with Nestling Growth and Fledging Success in Wild Passerines. Tartu, 2016, 113 p.
287. **Ann Kraut**. Conservation of Wood-Inhabiting Biodiversity – Semi-Natural Forests as an Opportunity. Tartu, 2016, 141 p.
288. **Tiit Örd**. Functions and regulation of the mammalian pseudokinase TRIB3. Tartu, 2016, 182. p.
289. **Kairi Käiro**. Biological Quality According to Macroinvertebrates in Streams of Estonia (Baltic Ecoregion of Europe): Effects of Human-induced Hydromorphological Changes. Tartu, 2016, 126 p.
290. **Leidi Laurimaa**. *Echinococcus multilocularis* and other zoonotic parasites in Estonian canids. Tartu, 2016, 144 p.
291. **Helerin Margus**. Characterization of cell-penetrating peptide/nucleic acid nanocomplexes and their cell-entry mechanisms. Tartu, 2016, 173 p.
292. **Kadri Runnel**. Fungal targets and tools for forest conservation. Tartu, 2016, 157 p.
293. **Urmo Võsa**. MicroRNAs in disease and health: aberrant regulation in lung cancer and association with genomic variation. Tartu, 2016, 163 p.
294. **Kristina Mäemets-Allas**. Studies on cell growth promoting AKT signaling pathway – a promising anti-cancer drug target. Tartu, 2016, 146 p.
295. **Janeli Viil**. Studies on cellular and molecular mechanisms that drive normal and regenerative processes in the liver and pathological processes in Dupuytren's contracture. Tartu, 2016, 175 p.
296. **Ene Kook**. Genetic diversity and evolution of *Pulmonaria angustifolia* L. and *Myosotis laxa sensu lato* (Boraginaceae). Tartu, 2016, 106 p.
297. **Kadri Peil**. RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*. Tartu, 2016, 113 p.
298. **Katrin Ruisu**. The role of RIC8A in mouse development and its function in cell-matrix adhesion and actin cytoskeletal organisation. Tartu, 2016, 129 p.
299. **Janely Pae**. Translocation of cell-penetrating peptides across biological membranes and interactions with plasma membrane constituents. Tartu, 2016, 126 p.
300. **Argo Ronk**. Plant diversity patterns across Europe: observed and dark diversity. Tartu, 2016, 153 p.
301. **Kristiina Mark**. Diversification and species delimitation of lichenized fungi in selected groups of the family Parmeliaceae (Ascomycota). Tartu, 2016, 181 p.
302. **Jaak-Albert Metsoja**. Vegetation dynamics in floodplain meadows: influence of mowing and sediment application. Tartu, 2016, 140 p.

303. **Hedvig Tamman**. The GraTA toxin-antitoxin system of *Pseudomonas putida*: regulation and role in stress tolerance. Tartu, 2016, 154 p.
304. **Kadri Pärtel**. Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. Tartu, 2016, 183 p.
305. **Maris Hindrikson**. Grey wolf (*Canis lupus*) populations in Estonia and Europe: genetic diversity, population structure and -processes, and hybridization between wolves and dogs. Tartu, 2016, 121 p.
306. **Polina Degtjarenko**. Impacts of alkaline dust pollution on biodiversity of plants and lichens: from communities to genetic diversity. Tartu, 2016, 126 p.
307. **Liina Pajusalu**. The effect of CO_2 enrichment on net photosynthesis of macrophytes in a brackish water environment. Tartu, 2016, 126 p.
308. **Stoyan Tankov**. Random walks in the stringent response. Tartu, 2016, 94 p.
309. **Liis Leitsalu**. Communicating genomic research results to population-based biobank participants. Tartu, 2016, 158 p.
310. **Richard Meitern**. Redox physiology of wild birds: validation and application of techniques for detecting oxidative stress. Tartu, 2016, 134 p.
311. **Kaie Lokk**. Comparative genome-wide DNA methylation studies of healthy human tissues and non-small cell lung cancer tissue. Tartu, 2016, 127 p.
312. **Mihhail Kurašin**. Processivity of cellulases and chitinases. Tartu, 2017, 132 p.
313. **Carmen Tali**. Scavenger receptors as a target for nucleic acid delivery with peptide vectors. Tartu, 2017, 155 p.
314. **Katarina Oganjan**. Distribution, feeding and habitat of benthic suspension feeders in a shallow coastal sea. Tartu, 2017, 132 p.
315. **Taavi Paal**. Immigration limitation of forest plants into wooded landscape corridors. Tartu, 2017, 145 p.
316. **Kadri Õunap**. The Williams-Beuren syndrome chromosome region protein WBSCR22 is a ribosome biogenesis factor. Tartu, 2017, 135 p.
317. **Riin Tamm**. In-depth analysis of factors affecting variability in thiopurine methyltransferase activity. Tartu, 2017, 170 p.
318. **Keiu Kask**. The role of RIC8A in the development and regulation of mouse nervous system. Tartu, 2017, 184 p.
319. **Tiia Möller**. Mapping and modelling of the spatial distribution of benthic macrovegetation in the NE Baltic Sea with a special focus on the eelgrass *Zostera marina* Linnaeus, 1753. Tartu, 2017, 162 p.
320. **Silva Kasela**. Genetic regulation of gene expression: detection of tissue- and cell type-specific effects. Tartu, 2017, 150 p.
321. **Karmen Süld**. Food habits, parasites and space use of the raccoon dog *Nyctereutes procyonoides*: the role of an alien species as a predator and vector of zoonotic diseases in Estonia. Tartu, 2017, p.
322. **Ragne Oja**. Consequences of supplementary feeding of wild boar – concern for ground-nesting birds and endoparasite infection. Tartu, 2017, 141 p.
323. **Riin Kont**. The acquisition of cellulose chain by a processive cellobiohydrolase. Tartu, 2017, 117 p.

324. **Liis Kasari**. Plant diversity of semi-natural grasslands: drivers, current status and conservation challenges. Tartu, 2017, 141 p.
325. **Sirgi Saar**. Belowground interactions: the roles of plant genetic relatedness, root exudation and soil legacies. Tartu, 2017, 113 p.
326. **Sten Anslan**. Molecular identification of Collembola and their fungal associates. Tartu, 2017, 125 p.
327. **Imre Taal**. Causes of variation in littoral fish communities of the Eastern Baltic Sea: from community structure to individual life histories. Tartu, 2017, 118 p.
328. **Jürgen Jalak**. Dissecting the Mechanism of Enzymatic Degradation of Cellulose Using Low Molecular Weight Model Substrates. Tartu, 2017, 137 p.
329. **Kairi Kiik**. Reproduction and behaviour of the endangered European mink (*Mustela lutreola*) in captivity. Tartu, 2018, 112 p.
330. **Ivan Kuprijanov**. Habitat use and trophic interactions of native and invasive predatory macroinvertebrates in the northern Baltic Sea. Tartu, 2018, 117 p.
331. **Hendrik Meister**. Evolutionary ecology of insect growth: from geographic patterns to biochemical trade-offs. Tartu, 2018, 147 p.
332. **Ilja Gaidutšik**. Irc3 is a mitochondrial branch migration enzyme in *Saccharomyces cerevisiae.* Tartu, 2018, 161 p.
333. **Lena Neuenkamp**. The dynamics of plant and arbuscular mycorrhizal fungal communities in grasslands under changing land use. Tartu, 2018, 241 p.
334. **Laura Kasak**. Genome structural variation modulating the placenta and pregnancy maintenance. Tartu, 2018, 181 p.
335. **Kersti Riibak**. Importance of dispersal limitation in determining dark diversity of plants across spatial scales. Tartu, 2018, 133 p.
336. **Liina Saar**. Dynamics of grassland plant diversity in changing landscapes. Tartu, 2018, 206 p.
337. **Hanna Ainelo**. Fis regulates *Pseudomonas putida* biofilm formation by controlling the expression of *lapA*. Tartu, 2018, 143 p.
338. **Natalia Pervjakova**. Genomic imprinting in complex traits. Tartu, 2018, 176 p.
339. **Andrio Lahesaare**. The role of global regulator Fis in regulating the expression of *lapF* and the hydrophobicity of soil bacterium *Pseudomonas putida*. Tartu, 2018, 124 p.
340. **Märt Roosaare**. *K*-mer based methods for the identification of bacteria and plasmids. Tartu, 2018, 117 p.
341. **Maria Abakumova**. The relationship between competitive behaviour and the frequency and identity of neighbours in temperate grassland plants. Tartu, 2018, 104 p.
342. **Margus Vilbas**. Biotic interactions affecting habitat use of myrmecophilous butterflies in Northern Europe. Tartu, 2018, 142 p.

343. **Liina Kinkar**. Global patterns of genetic diversity and phylogeography of *Echinococcus granulosus* sensu stricto – a tapeworm species of significant public health concern. Tartu, 2018, 147 p.
344. **Teivi Laurimäe**. Taxonomy and genetic diversity of zoonotic tapeworms in the species complex of *Echinococcus granulosus* sensu lato. Tartu, 2018, 143 p.
345. **Tatjana Jatsenko**. Role of translesion DNA polymerases in mutagenesis and DNA damage tolerance in Pseudomonads. Tartu, 2018, 216 p.
346. **Katrin Viigand**. Utilization of α-glucosidic sugars by *Ogataea* (*Hansenula*) *polymorpha.* Tartu, 2018, 148 p.
347. **Andres Ainelo**. Physiological effects of the *Pseudomonas putida* toxin grat. Tartu, 2018, 146 p.
348. **Killu Timm**. Effects of two genes (DRD4 and SERT) on great tit (*Parus major*) behaviour and reproductive traits. Tartu, 2018, 117 p.
349. **Petr Kohout**. Ecology of ericoid mycorrhizal fungi. Tartu, 2018, 184 p.
350. **Gristin Rohula-Okunev**. Effects of endogenous and environmental factors on night-time water flux in deciduous woody tree species. Tartu, 2018, 184 p.
351. **Jane Oja**. Temporal and spatial patterns of orchid mycorrhizal fungi in forest and grassland ecosystems. Tartu, 2018, 102 p.
352. **Janek Urvik**. Multidimensionality of aging in a long-lived seabird. Tartu, 2018, 135 p.
353. **Lisanna Schmidt**. Phenotypic and genetic differentiation in the hybridizing species pair *Carex flava* and *C. viridula* in geographically different regions. Tartu, 2018, 133 p.
354. **Monika Karmin**. Perspectives from human Y chromosome – phylogeny, population dynamics and founder events. Tartu, 2018, 168 p.
355. **Maris Alver**. Value of genomics for atherosclerotic cardiovascular disease risk prediction. Tartu, 2019, 148 p.
356. **Lehti Saag**. The prehistory of Estonia from a genetic perspective: new insights from ancient DNA. Tartu, 2019, 171 p.
357. **Mari-Liis Viljur**. Local and landscape effects on butterfly assemblages in managed forests. Tartu, 2019, 115 p.
358. **Ivan Kisly**. The pleiotropic functions of ribosomal proteins eL19 and eL24 in the budding yeast ribosome. Tartu, 2019, 170 p.
359. **Mikk Puustusmaa**. On the origin of papillomavirus proteins. Tartu, 2019, 152 p.
360. **Anneliis Peterson**. Benthic biodiversity in the north-eastern Baltic Sea: mapping methods, spatial patterns, and relations to environmental gradients. Tartu, 2019, 159 p.
361. **Erwan Pennarun**. Meandering along the mtDNA phylogeny; causerie and digression about what it can tell us about human migrations. Tartu, 2019, 162 p.

362. **Karin Ernits**. Levansucrase Lsc3 and endo-levanase BT1760: characterization and application for the synthesis of novel prebiotics. Tartu, 2019, 217 p.
363. **Sille Holm**. Comparative ecology of geometrid moths: in search of contrasts between a temperate and a tropical forest. Tartu, 2019, 135 p.
364. **Anne-Mai Ilumäe**. Genetic history of the Uralic-speaking peoples as seen through the paternal haplogroup N and autosomal variation of northern Eurasians. Tartu, 2019, 172 p.
365. **Anu Lepik**. Plant competitive behaviour: relationships with functional traits and soil processes. Tartu, 2019, 152 p.
366. **Kunter Tätte**. Towards an integrated view of escape decisions in birds under variable levels of predation risk. Tartu, 2020, 172 p.
367. **Kaarin Parts**. The impact of climate change on fine roots and root-associated microbial communities in birch and spruce forests. Tartu, 2020, 143 p.
368. **Viktorija Kukuškina**. Understanding the mechanisms of endometrial receptivity through integration of 'omics' data layers. Tartu, 2020, 169 p.
369. **Martti Vasar**. Developing a bioinformatics pipeline gDAT to analyse arbuscular mycorrhizal fungal communities using sequence data from different marker regions. Tartu, 2020, 193 p.
370. **Ott Kangur**. Nocturnal water relations and predawn water potential disequilibrium in temperate deciduous tree species. Tartu, 2020, 126 p.
371. **Helen Post**. Overview of the phylogeny and phylogeography of the Y-chromosomal haplogroup N in northern Eurasia and case studies of two linguistically exceptional populations of Europe – Hungarians and Kalmyks. Tartu, 2020, 143 p.
372. **Kristi Krebs**. Exploring the genetics of adverse events in pharmacotherapy using Biobanks and Electronic Health Records. Tartu, 2020, 151 p.
373. **Kärt Ukkivi**. Mutagenic effect of transcription and transcription-coupled repair factors in *Pseudomonas putida.* Tartu, 2020, 154 p.
374. **Elin Soomets**. Focal species in wetland restoration. Tartu, 2020, 137 p.
375. **Kadi Tilk**. Signals and responses of ColRS two-component system in *Pseudomonas putida.* Tartu, 2020, 133 p.
376. **Indrek Teino**. Studies on aryl hydrocarbon receptor in the mouse granulosa cell model. Tartu, 2020, 139 p.
377. **Maarja Vaikre**. The impact of forest drainage on macroinvertebrates and amphibians in small waterbodies and opportunities for cost-effective mitigation. Tartu, 2020, 132 p.
378. **Siim-Kaarel Sepp**. Soil eukaryotic community responses to land use and host identity. Tartu, 2020, 222 p.
379. **Eveli Otsing**. Tree species effects on fungal richness and community structure. Tartu, 2020, 152 p.
380. **Mari Pent**. Bacterial communities associated with fungal fruitbodies. Tartu, 2020, 144 p.

381. **Einar Kärgenberg**. Movement patterns of lithophilous migratory fish in free-flowing and fragmented rivers. Tartu, 2020, 167 p.
382. **Antti Matvere**. The studies on aryl hydrocarbon receptor in murine granulosa cells and human embryonic stem cells. Tartu, 2021, 163 p.
383. **Jhonny Capichoni Massante**. Phylogenetic structure of plant communities along environmental gradients: a macroecological and evolutionary approach. Tartu, 2021, 144 p.
384. **Ajai Kumar Pathak**. Delineating genetic ancestries of people of the Indus Valley, Parsis, Indian Jews and Tharu tribe. Tartu, 2021, 197 p.
385. **Tanel Vahter**. Arbuscular mycorrhizal fungal biodiversity for sustainable agroecosystems. Tartu, 2021, 191 p.
386. **Burak Yelmen**. Characterization of ancient Eurasian influences within modern human genomes. Tartu, 2021, 134 p.
387. **Linda Ongaro**. A genomic portrait of American populations. Tartu, 2021, 182 p.
388. **Kairi Raime**. The identification of plant DNA in metagenomic samples. Tartu, 2021, 108 p.
389. **Heli Einberg**. Non-linear and non-stationary relationships in the pelagic ecosystem of the Gulf of Riga (Baltic Sea). Tartu, 2021, 119 p.
390. **Mickaël Mathieu Pihain**. The evolutionary effect of phylogenetic neighbourhoods of trees on their resistance to herbivores and climatic stress. Tartu, 2022, 145 p.
391. **Annika Joy Meitern**. Impact of potassium ion content of xylem sap and of light conditions on the hydraulic properties of trees. Tartu, 2022, 132 p.
392. **Elise Joonas**. Evaluation of metal contaminant hazard on microalgae with environmentally relevant testing strategies. Tartu, 2022, 118 p.
393. **Kreete Lüll**. Investigating the relationships between human microbiome, host factors and female health. Tartu, 2022, 141 p.
394. **Triin Kaasiku**. A wader perspective to Boreal Baltic coastal grasslands: from habitat availability to breeding site selection and nest survival. Tartu, 2022, 141 p.
395. **Meeli Alber**. Impact of elevated atmospheric humidity on the structure of the water transport pathway in deciduous trees. Tartu, 2022, 170 p.
396. **Ludovica Molinaro**. Ancestry deconvolution of Estonian, European and Worldwide genomic layers: a human population genomics excavation. Tartu, 2022, 138 p.
397. **Tina Saupe**. The genetic history of the Mediterranean before the common era: a focus on the Italian Peninsula. Tartu, 2022, 165 p.
398. **Mari-Ann Lind**. Internal constraints on energy processing and their consequences: an integrative study of behaviour, ornaments and digestive health in greenfinches. Tartu, 2022, 137 p.
399. **Markus Valge**. Testing the predictions of life history theory on anthropometric data. Tartu, 2022, 171 p.
400. **Ants Tull**. Domesticated and wild mammals as reservoirs for zoonotic helminth parasites in Estonia. Tartu, 2022, 152 p.

401. **Saleh Rahimlouye Barabi**. Investigation of diazotrophic bacteria association with plants. Tartu, 2022, 137 p.
402. **Farzad Aslani**. Towards revealing the biogeography of belowground diversity. Tartu, 2022, 124 p.
403. **Nele Taba**. Diet, blood metabolites, and health. Tartu, 2022, 163 p.
404. **Katri Pärna**. Improving the personalized prediction of complex traits and diseases: application to type 2 diabetes. Tartu, 2022, 190 p.
405. **Silva Lilleorg**. Bacterial ribosome heterogeneity on the example of bL31 paralogs in *Escherichia coli.* Tartu, 2022, 189 p.
406. **Oliver Aasmets**. The importance of microbiome in human health. Tartu, 2022, 123 p.
407. **Henel Jürgens**. Exploring post-translational modifications of histones in RNA polymerase II-dependent transcription. Tartu, 2022, 147 p.
408. **Mari Tagel**. Finding novel factors affecting the mutation frequency: a case study of tRNA modification enzymes TruA and RluA. Tartu, 2022, 176 p.
409. **Marili Sell**. The impact of environmental change on ecophysiology of hemiboreal tree species – acclimation mechanisms in belowground. Tartu, 2022, 163 p.
410. **Kaarin Hein**. The hissing behaviour of Great Tit (*Parus major*) females reflects behavioural phenotype and breeding success in a wild population. Tartu, 2022, 96 p.
411. **Maret Gerz**. The distribution and role of mycorrhizal symbiosis in plant communities. Tartu, 2022, 206 p.
412. **Kristiina Nõomaa**. Role of invasive species in brackish benthic community structure and biomass changes. Tartu, 2023, 151 p.
413. **Anton Savchenko**. Taxonomic studies in Dacrymycetes: *Cerinomyces* and allied taxa. Tartu, 2023, 181 p.
414. **Ahto Agan**. Interactions between invasive pathogens and resident mycobiome in the foliage of trees. Tartu, 2023, 155 p.
415. **Diego Pires Ferraz Trindade**. Dark diversity dynamics linked to global change: taxonomic and functional perspective. Tartu, 2023, 134 p.
416. **Madli Jõks**. Biodiversity drivers in oceanic archipelagos and habitat fragments, explored by agent-based simulation models. Tartu, 2023, 116 p.
417. **Ciara Baines**. Adaptation to oncogenic pollution and natural cancer defences in the aquatic environment. Tartu, 2023, 164 p.
418. **Rain Inno**. Placental transcriptome and miRNome in normal and complicated pregnancies. Tartu, 2023, 145 p.
419. **Daniyal Gohar**. Diversity, genomics, and potential functions of fungus-inhabiting bacteria. Tartu, 2023, 138 p.
420. **Sirli Rosendahl**. Fitness effects of chromosomal toxin-antitoxin systems in *Pseudomonas putida.* Tartu, 2023, 154 p.
421. **Mathilde Frédérique E. André**. New Guinea, a hotspot for Human evolution: settlement history and adaptation in northern Sahul. Tartu, 2023, 202 p.

422. **Vlad-Julian Piljukov**. Biochemical characterization of Irc3 helicase. Tartu, 2023, 137 p.
423. **Gerli Albert**. Carbon use strategies of macrophyte communities in the northeastern Baltic Sea: implications for a high CO_2 environment. Tartu, 2023, 128 p.
424. **Mariann Koel**. The molecular interactions between trophoblast and endometrial cells in embryo implantation. Tartu, 2023, 171 p.
425. **Robin Gielen**. Diversity and ecological role of pathogenic fungi in insect populations. Tartu, 2023, 139 p.
426. **Kaspar Reier**. Quantity, stability and disparity of ribosomal components in *Escherichia coli* stationary phase. Tartu, 2023, 151 p.
427. **Linda Rusalepp**. The impact of environmental drivers and competition on phenolic metabolite profiles in hybrid aspen and silver birch. Tartu, 2023, 153 p.
428. **Eliisa Pass**. The effect of managed forest-wetland landscapes on forest grouse and nest predation. Tartu, 2023, 115 p.
429. **Sanni Färkkilä**. Methods for studying plant-fungal interactions – reflecting on the old, the new and the upcoming. Tartu, 2024, 147 p.
430. **Maarja Jõeloo**. Advances in microarray-based copy number variation discovery and phenotypic associations. Tartu, 2024, 209 p.
431. **Natàlia Pujol Gualdo**. Decoding genetic associations of female reproductive health traits. Tartu, 2024, 205 p.
432. **Sirelin Sillamaa**. The role of helicases Hmi1 and Irc3 in yeast mitochondrial DNA maintenance. Tartu, 2024, 189 p.
433. **Iris Reinula**. Genetic variation of grassland plants in changing landscapes. Tartu, 2024, 201 p.
434. **Vi Ngan Tran**. The cellular dynamics and epithelial morphogenesis in *Drosophila* wing development. Tartu, 2024, 158 p.
435. **Slendy Julieth Rodríguez Alarcón**. Intraspecific trait diversity in plants: characterizing effects of trait variation on community assembly and ecosystem functioning. Tartu, 2024, 129 p.
436. **Arun Kumar Devarajan**. Microbes and climate change: insights from plant-microbe interactions in rice phyllosphere and soil microbiomes in subarctic grasslands. Tartu, 2024, 224 p.
437. **Leonard Owuraku Opare**. Rearing density effects on a commercially important insect species. Tartu, 2024, 145 p.
438. **Siqiao Liu**. The effect of anthropogenic disturbance on soil fungal communities. Tartu, 2024, 172 p.
439. **Kertu Liis Krigul**. The gut microbiome at the interface of human health and disease. Tartu, 2024, 158 p.
440. **Danat Yermakovich**. The evolutionary history of complex traits: implications of archaic admixture. Tartu, 2024, 153 p.
441. **Yiming Meng**. Plant mycorrhizal type and status in the global flora. Tartu, 2024, 200 p.

442. **Iryna Yatsiuk**. Evolution, species delimitation and diversity in myxomycetes: *Arcyria* and allied genera. Tartu, 2024, 193 p.
443. **Daniela León Velandia**. Mycorrhizal trait distribution and composition in plant communities under natural gradients. Tartu, 2024, 121 p.
444. **Bruno Paganeli**. Dark diversity methods for prioritization of areas and species in nature conservation. Tartu, 2024, 155 p.