Exploring the Association of Maternal Covid-19 Infection and Epigenetic Regulation of Metabolic Pathways in Newborn Children

by

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Abstract

Background: SARS-CoV-2 infection during pregnancy has emerged as a significant concern due to its potential implications for fetal health. Viral infections can induce inflammatory responses and immune activation in pregnant individuals, which may harm the developing fetus through epigenetic alterations, potentially influencing gene expression patterns and developmental trajectories even without vertical transmission. While much remains to be understood about the specific effects of SARS-CoV-2 on pregnancy, previous research on other viral infections, such as influenza, Zika virus, and cytomegalovirus, has highlighted the potential for adverse outcomes, including asthma, metabolic disease, and mental illness.

Methods: We used a gene expression dataset accessed on the Gene Expression Omnibus database (GSE 165193), including 14,748 cells from cases, and 11,222 cells from controls. In this case-control study, droplet-based single-cell RNA-sequencing (SC-RNA Seq) and T-cell receptor sequencing were performed on Cord Blood Mononuclear Cells (CBMCs) involving three-term gestation infants (>37 weeks) born to mothers with mild COVID-19 symptoms (cases) and three infants born to uninfected mothers (controls). Initially the differential expression of the "Reactome_Epigenetic_Regulation_of_Gene_Expression" collection was evaluated employing the linear combination test (LCT) on 25,970 cells including 18 types to discern epigenetic modulation comparing cases vs. controls. Subsequently, differential expression of biological pathway collection of Kyoto Encyclopedia of Genes and Genomes (KEGG) in CBMCs of COVID-19-infected mothers exhibiting epigenetic modulation explored by performing Linear Combination Test (LCT).

Results: Our analysis revealed differential expression of genes associated with epigenetic regulation in specific cell types of cord blood from infants born to COVID-19-infected mothers compared to controls. Subsequent analysis using KEGG collections on SARS-CoV-2 infected samples identified disturbed biological pathways related to amino acid and lipid biosynthesis and metabolism. These pathways have previously been implicated in metabolic diseases, particularly cardiovascular disease.

Conclusions: Maternal COVID-19 infection during pregnancy is associated with epigenetic modulation compared to healthy pregnant mothers. The identification of disturbed biological pathways in COVID-19-infected mothers suggests a link between maternal infection, epigenetic modulation, and the development of metabolic diseases in offspring. These findings underscore the importance of prenatal care and highlight potential targets for preventive interventions to mitigate long-term metabolic disease risk, specifically cardiovascular diseases (CVD) in neonates born to COVID-19-infected-mothers.

Preface

This thesis represents the original research conducted by Fatemeh Nezarat under the guidance of Dr. Irina Dinu. I was responsible for conceptualizing the study, identifying public datasets, designing figures and tables, conducting literature reviews, and taking the lead in drafting conference abstracts and presentations. Professor Irina Dinu provided comprehensive support throughout all steps of the project, contributing to its conceptualization and manuscript formulation. The data analysis presented in Chapter 3 was a collaborative effort involving Nastaran Hajizadeh Bastani and Sara Khademioureh. Additionally, Professor Saumyadipta Pyne played a supervisory role in the project, providing guidance on conference manuscript authorship and contributing to data analysis and interpretation of the results. Figure 1. 1 is extracted from the "Reactome", publicly available pathway database. Figure 2. 1, Table 2. 1 and Figure 2. 3 is extracted from the main paper we used the data for our research project (GSE 165193) (Matute et al., 2022).

Portions of this research have been presented as detailed below:

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Chapter 1: Coronavirus

Discovered over half a century ago, coronaviruses, characterized by their distinctive crown-shaped morphology and affiliation with the Coronaviridae family, exhibit the capacity to infect a diverse array of organisms, encompassing humans, and induce acute and chronic respiratory, enteric, and central nervous system (CNS) pathologies. Their classification into four distinct genera (α, β, γ, δ) stems from serological cross-reactivity (Malik, 2020; Tyrrell & Bynoe, 1966; Weiss & Navas-Martin, 2005).

1.1 Human Coronaviruses (HCoVs)

Within the four designated coronavirus genera (α, β, γ, δ), human coronaviruses (HCoVs) are categorized within the α-CoV group (including HCoV-229E and NL63) and the β-CoV group (encompassing MERS-CoV, SARS-CoV, HCoV-OC43, and HCoV-HKU1). The low-pathogenic human species include 229E, OC43, NL63, and HKU1 which cause mild upper respiratory tract infections (URTIs) such as the common cold, whereas SARS-CoV, MERS-CoV, and SARS-CoV-2 are highly pathogenic and cause severe illness (Malik, 2020).

Following the identification of a novel human beta coronavirus in 2002–2003, along with civet cats serving as its intermediate host in Guangdong Province, China, a paradigm shift occurred regarding the association of coronaviruses with severe human illnesses. SARS infection was documented in 29 countries across North America, South America, Europe, and Asia. The Centers for Disease Control and Prevention (CDC) documented over 8,000 cases and near 800 fatalities during the SARS epidemic, further amplifying the significance of coronaviruses in public consciousness (Kahn & McIntosh, 2005; Malik, 2020; Knobler et al., 2004).

Since the identification of SARS-CoV, five additional human coronaviruses associated with respiratory illness have been reported (Table 1. 1). HCoV-NL63 was first detected in a Dutch infant aged seven months who presented with bronchiolitis and conjunctivitis (van der Hoek et al., 2004). A senior citizen suffering from pneumonia was the source of HKU1's isolation (Woo et al., 2005). MERS-CoV originated from a severe pneumonia case in a 60-year-old Saudi Arabian man and later spread to approximately 1800 individuals, with a fatality rate of around 35% (Mackay &

Arden, 2015; Widagdo et al., 2017). The latest coronavirus, SARS-CoV-2, responsible for the COVID-19 pandemic, was first identified in Wuhan, China, in December 2019 (Malik, 2020).

Table 1. 1 Human Coronaviruses, Location, Year of Discovery

Virus	***Location***	***Year***
SARS	China	2003
HCoV-NL63	Netherlands	2004
*HCoV-NH**	New Haven, CT	2005
HKU1	Hong Kong	2005
MERS-CoV	Saudi Arabia	2012
SARS-CoV-2	China	2019

* HCoV-NH is the name given to the variant of NL-63 that was independently isolated in New Haven, Connecticut.

1.1.1 Coronavirus Disease 2019 (COVID-19): Insights into Epidemiology, Clinical Manifestations and Complications

The emergence of the COVID-19 pandemic was officially reported to the WHO on December 31, 2019, following the detection of numerous pneumonia cases in Wuhan City, located in Hubei Province, China, with an unknown cause. Analysis of genome sequencing performed on respiratory secretions from affected individuals revealed a virus closely resembling SARS-CoV, which belongs to the Sarbecovirus subgenus within the β-coronavirus family, as the causative agent of the outbreak. The World Health Organization (WHO) officially named the novel coronavirus-induced pneumonia "Coronavirus Disease 2019" (COVID-19) on February 11, 2020, marking a significant moment in a global health crisis. The situation escalated further when the WHO declared COVID-19 a global pandemic in March 2020, following a surge in confirmed cases surpassing 100,000 worldwide. The rapid surge in COVID-19 cases during the early stages of the pandemic was attributed, in part, to the highly contagious nature of the virus and the limited availability of diagnostic tools and knowledge about the novel coronavirus and preventative measures (Hui et al., 2020; Malik, 2020; World Health Organization, 2020; World Health Organization, 2; 2020; J. T. Wu et al., 2020). The case fatality rate of COVID-19 varies

significantly depending on the country, ranging from 0 to over 20%. This variability is often linked to the presence of comorbidities such as diabetes and cardiovascular diseases, which can exacerbate the severity of the illness and increase the risk of mortality (Rahman et al., 2021; Sorci et al., 2020). Although SARS-CoV-2 demonstrates a lower fatality rate in comparison to SARS-CoV or MERS-CoV, its high transmissibility sets it apart. By February 2024, the World Health Organization (WHO) had reported approximately 775 million confirmed cases of COVID-19 worldwide, with over 6 million fatalities across 235 countries. (WHO, 2024). As of now, the Virus Evolution Working Group of the World Health Organization has pinpointed several variants of SARS-CoV-2, notably including Alpha (B.1.1.7 lineage), Beta (B.1.351 lineage), Gamma (P.1 lineage), Delta (B.1.617.2 lineage), and Omicron (B.1.1.529 lineage) variants, all classified as variants of concern (VOC) (Chen et al., 2023; Wu et al., 2022). The World Health Organization officially declared the end of the pandemic on May 5, 2023; however, it acknowledges that COVID-19 remains an enduring and significant health challenge. Given the ongoing emergence of new variants of the SARS-CoV-2 virus, there is a critical imperative to expand research endeavors to comprehensively understand the distinct impacts of this virus (WHO, 2023).

During the incubation period, individuals infected with COVID-19 may experience a gradual respiratory response triggered by the virus. This period, characterized by the presence of the virus in the throat or nose before symptom onset, typically lasts 1 to 14 days. However, the emergence of variants has led to slight variations in the average length of the incubation period, with estimates ranging from 5.00 to 3.42 days (Wu et al., 2022). Following the incubation period, individuals infected with COVID-19 typically exhibit a range of symptoms often resembling other respiratory illnesses, making diagnosis challenging, including fever, respiratory symptoms such as cough and shortness of breath, dry cough, dyspnea, viral pneumonia, fatigue, and loss of appetite. Additionally, gastrointestinal symptoms such as diarrhea and vomiting may occur less frequently. In severe cases, patients may experience complications such as severe acute respiratory syndrome, heart failure, renal failure, hypercoagulation, acute respiratory distress syndrome (ARDS), septic shock, and death (Guo et al., 2020; Habas et al., 2020; Jin et al., 2020).

1.1.2 SARS CoV-2: Molecular Features and Routes of Transmission

SARS-CoV-2, a member of the Betacoronavirus genus, possesses distinctive characteristics, being an enveloped, positive-sense, single-stranded RNA virus. Its genomic sequences share approximately 80% identity with SARS-CoV and around 50% with MERS-CoV (Lu et al., 2020). ACE2 serves as a cell entry receptor for SARS-CoV-2, initially identified in 2003 as the receptor for SARS-CoV, highlighting its multifaceted role in normal physiology and viral pathogenesis. (Lan et al., 2020; Li et al., 2003; Shang, Ye, et al., 2020; Walls et al., 2020; Wang et al., 2020). It is essential to highlight that the catalytic function of ACE2 is distinct from its role as a cell entry receptor for viruses like SARS-CoV-2. Inhibitors aimed at the catalytic site of ACE2 do not affect the virus-binding process, underscoring the complex interplay between receptor function and viral entry mechanisms. (Li et al., 2005). The genome of SARS-CoV-2 consists of 14 open reading frames (ORFs), with about two-thirds of its sequence devoted to encoding 16 non-structural proteins (nsp1–16), crucial components of the replicase complex. The remaining one-third of the genome encodes nine accessory proteins (ORF) and four structural proteins: nucleocapsid (N), membrane (M), envelope (E), and spike (S) proteins (Perlman & Netland, 2009; Zhang & Holmes, 2020). The entry of viral particles into host cells, involving attachment to the cell membrane and subsequent fusion, is facilitated by the class I viral fusion S glycoprotein. Assembled as a homotrimer, the S protein is integrated into the virion membrane in multiple copies, giving it a distinctive crown-like appearance. Within virus-producing cells, the S protein of SARS-CoV-2 undergoes cleavage by proprotein convertases. This results in the mature virion's S protein consisting of two non-covalently associated subunits: the S1 subunit, responsible for ACE2 binding, and the S2 subunit, which anchors the S protein to the membrane. The S1 subunit exhibits a structure comprising four domains: the amino-terminal domain (NTD), the receptor-binding domain (RBD), and two carboxy-terminal domains (CTD1 and CTD2). Mutations within the RBD of the Spike protein of SARS-CoV-2 have been identified as critical factors enabling additional interactions with ACE2. These mutations enhance the binding affinity between the virus and its host receptor, potentially leading to increased infectivity compared to SARS-CoV (Wrapp et al., 2020). The three receptor-binding domains (RBDs) at the top of the S protein trimer oscillate between two distinct states: an 'up' position that facilitates receptor accessibility and a 'down' position that limits receptor interaction (Song et al., 2018; Zhou et al., 2020). Moreover, the S2 subunit undergoes significant conformational changes to facilitate membrane fusion upon

infecting a new cell (Fehr & Perlman, 2015; Hoffmann et al., 2020; Shang, Wan, et al., 2020). When the virus engages ACE2, it exposes the internal S2' site of the S2 subunit. Cleavage of the S2' site—either by transmembrane protease, serine 2 (TMPRSS2) on the cell surface or by cathepsin L in the endosomal compartment following ACE2-mediated endocytosis—results in the release of the fusion peptide initiating the formation of a fusion pore (Glowacka et al., 2011; Huang et al., 2006; Jackson et al., 2022; Shulla et al., 2011; Simmons et al., 2005). The efficiency of viral entry relies heavily on TMPRSS2 expression, as SARS-CoV can still infiltrate cells even when ACE2 levels are low, provided TMPRSS2 is present (Shulla et al., 2011). Once inside the host cytosol, the viral genome is translated into viral replicase proteins through ORF1a and ORF1b. These proteins are subsequently cleaved into individual non-structural proteins (nsps) by both host and viral proteases. The replicase components remodel the endoplasmic reticulum (ER) to generate double-membrane vesicles (DMVs). These DMVs serve as sites for viral replication, where both genomic and subgenomic RNAs (sgRNA) undergo duplication. Post-replication, the sgRNAs are translated into accessory and viral structural proteins, vital for assembling new virus particles (Harrison et al., 2020; Snijder et al., 2006; Wu & Brian, 2010).

Human coronaviruses are mainly transmitted through respiratory droplets, although alternative routes such as aerosols, direct contact with contaminated surfaces, and fecal-oral transmission were also observed during the SARS epidemic (Otter et al., 2016; Yu et al., 2004).

Animal-to-Human Transmission

Current research suggests that the transmission of COVID-19 likely began in bats but may have been transferred to humans through intermediate animals, possibly originating from the seafood market in Wuhan City, Hubei province, China. Studies conducted by Xiao et al. highlight the essential role of an intermediary host in the transmission of SARS-CoV-2 to humans, given that coronaviruses originating from bats seldom infect humans directly (Xiao et al., 2020).

Human-to-Human Transmission

The principal transmission mechanism from person to person is currently known to involve respiratory droplets expelled by infected individuals. Thus, actions like coughing and sneezing have the potential to disseminate SARS-CoV-2, thereby posing a risk of infection to those who are not yet infected (Ather et al., 2020; Carlos et al., 2020). Additionally, there is now substantiated evidence supporting the transmission of SARS-CoV-2 by individuals who exhibit no symptoms (asymptomatic) or are in the pre-symptomatic stage, diverging from the transmission dynamics observed for SARS-CoV (Zou, et al., 2020).

Hospitals, like in the case of MERS-CoV, are recognized as significant sources of secondary transmission for SARS-CoV-2 due to the accumulation of infected individuals within their premises. Particularly concerning COVID-19, the presence of viral contamination in hospital wards where patients receive treatment has been identified as an additional route of transmission. A recent study by Santarpia et al. demonstrated the detection of SARS-CoV-2 in various everyday items within wards housing COVID-19-positive patients, as well as in air samples collected within hospital environments (Santarpia et al., 2020, Cai et al., 2020; Drosten et al., 2014; Sharma et al., 2021)

Maternal Transmission or Vertical Transmission

Pregnancy is acknowledged as a critical period marked by notable immune and physiological adjustments to accommodate a genetically distinct fetus. Physiological adaptations encompass reduced lung capacity, heightened oxygen consumption, and adjustments in heart rate. Prior research has illustrated that changes in the immune system during pregnancy, favoring a dominance of the T-helper 2 (Th2) system, designed to protect the fetus, can render the mother more susceptible to viral infections. This is because viral infections are typically controlled more effectively by the Th1 system, highlighting a vulnerability in pregnant individuals (Dashraath et al., 2020). Pregnancy heightens the susceptibility to influenza infection, particularly among pregnant women with underlying health conditions and during the third trimester of gestation (Rasmussen et al., 2012). The severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) epidemics have been notably severe,

resulting in a high mortality rate among infected pregnant women, with approximately one-third of cases resulting in fatalities (Alfaraj et al., 2019; Wong et al., 2004). According to a systematic review including 435 studies, pregnant women infected with SARS-CoV-2 seem to face an increased risk of severe illness, as evidenced by higher rates of hospitalization and admission to intensive care units (ICUs) compared to the general population. Existing comorbidities, non-white ethnicity, chronic hypertension, pre-existing diabetes, advanced maternal age, and high body mass index are all identified as risk factors associated with severe COVID-19 outcomes during pregnancy. Pregnant individuals with COVID-19 are more prone to delivering prematurely and face a heightened risk of maternal mortality compared to those without the virus. Infants born to mothers with COVID-19 are at an increased likelihood of requiring admission to the neonatal intensive care unit (NICU) (Allotey et al., 2020).

In a systematic review and meta-analysis encompassing 69 studies, which reported on over 54,413 pregnancies affected by SARS-CoV-2 and resulted in the delivery of more than 30,840 newborns from infected mothers, analysis of tested samples showed that over 800 neonates tested positive for SARS-CoV-2. These findings suggest the potential for vertical transmission of SARS-CoV-2 from mothers infected with COVID-19 (Musa et al., 2021). Vertical transmission (VT) or mother-to-child transmission can transpire through diverse pathways, including the transplacental conveyance of microorganisms during gestation, exposure to blood and vaginal secretions during parturition, and via breastfeeding. Several determinants, such as maternal immunity, viral load in the placenta, and obstetric factors like preterm labor, low birth weight, fetal anomalies, and abortion, are strongly associated with an escalated likelihood of vertical transmission (VT). After delivery, exposure to infected caregivers emerges as the primary source of SARS-CoV-2 contagion in neonates. Breastfeeding has not been linked to an augmented risk of delayed postnatal transmission, delineated as transmission occurring beyond 72 hours of life. Nevertheless, refraining from segregating neonates from infected mothers has been correlated with an elevated risk of delayed postnatal transmission. (Chambers et al., 2020; Groß et al., 2020; Kimberlin & Puopolo, 2021; Wu et al., 2020). The placental membrane serves as a barrier, segregating maternal and fetal blood circulation. Despite this protective function, certain viruses, and bacteria such as Zika, CMV, and rubella, possess the ability to breach this barrier, leading to direct adverse effects on the fetus. However, the precise mechanisms facilitating this traversal

remain unclear (Yazigi et al., 2017). While SARS-CoV-2 has been observed to transmit to the fetus through the placenta, like SARS, such transmission appears to be relatively rare (Chen et al., 2020; Li et al., 2020, Murphy, 2020; Woodward, 2020; Barcelos et al., 2021; Jeganathan et al., 2020; Musa et al., 2021). Various factors may account for the apparent low rate of intrauterine transmission. One possible explanation is a potential correlation between decreased expression levels of ACE2 and TMPRSS2, both necessary for SARS-CoV-2 entry into placental cells, and the observed low rate of vertical transmission (Dashraath et al., 2020; Levy et al., 2008; Prochaska et al., 2020). Nevertheless, owing to their size, IgM antibodies cannot traverse the placenta and typically emerge in circulation 3–7 days following infection. Consequently, detecting IgM in cord blood samples from newborns indicates a potential intrauterine infection. Dong et al. documented instances of newborns with heightened IgM levels despite negative SARS-CoV-2 tests, underscoring that while vertical transmission is infrequent, it does happen (Dong et al., 2020; Gee et al., 2021; Mirbeyk et al., 2021; Zeng et al., 2020). Although neonates may have a low likelihood of acquiring a SARS-CoV-2 infection, the maternal inflammatory response linked to it could pose a threat to their overall health in the long term (Kleeman et al., 2022).

1.2 Infection During Pregnancy and Fetal Health

Previous research has shown that maternal viral infections during pregnancy, even if they do not vertically transmit to the child, can lead to long-term effects on the infant. These effects may include aberrant development of the nervous or immune systems due to maternal immunological activation during pregnancy (Abu-Raya et al., 2016; Al-Haddad, Jacobsson, et al., 2019; Al-Haddad, Oler, et al., 2019; Apostol et al., 2020; Matute et al., 2022). Severe infectious teratogens, such as those encompassed by the acronym TORCH (Toxoplasma gondii, rubella virus, cytomegalovirus, herpes simplex virus), can result in catastrophic structural anomalies in the fetal brain, including anencephaly, ventriculomegaly, deafness, and ocular injury (Al-Haddad, Oler, et al., 2019). A study by Benjamin et al. followed 179,152 children for 41 years and found that maternal hospitalization due to infectious disease increased the risk of autism and depression in offspring. Additionally, research has demonstrated a higher likelihood of schizophrenia hospitalization among individuals born to mothers who were pregnant during the peak of the 1957 influenza epidemic compared to those born before the epidemic. Furthermore, a meta-analysis of

nearly 40,000 cases of autism spectrum disorder (ASD) revealed an elevated risk of ASD after prenatal exposure to infection, mainly when the mother required hospitalization due to the infection (Al-Haddad, Jacobsson, et al., 2019; Brown et al., 2001; Canetta et al., 2014; Jiang et al., 2016; Limperopoulos et al., 2008; Machón et al., 1997; Mednick et al., 1988). Maternal genitourinary infections increase inflammatory responses in maternal and fetal tissues. Infants born to mothers who experienced a urinary tract infection (UTI) during pregnancy appear to face a heightened likelihood of developing various lifelong health conditions, including neural and cognitive issues and asthma (Bavaresco et al., 2019; Leviton et al., 2016; Fichorova et al., 2015). Thus, the consequences of maternal infection on fetal development stand as a pivotal concern in the discourse on infections during pregnancy. Even in cases where direct fetal infection does not occur, maternal SARS-CoV-2 infection can still impact fetal development.

1.3 Statement of Problem

Despite recognizing pregnant women as a high-risk group for SARS-CoV-2 infection due to physiological changes, hormonal fluctuations, and compromised immunity, our understanding of the virus's precise impact on fetal development and long-term health outcomes remains limited. Current literature lacks comprehensive data on the short-term and long-term implications of maternal COVID-19 infection on newborns, particularly regarding epigenetic regulation of metabolic pathways. This knowledge gap hampers our ability to devise effective clinical management strategies to protect maternal and fetal health. Therefore, there is a need for further research to investigate the association between maternal COVID-19 infection and epigenetic regulation of metabolic pathways in newborn children. Addressing this gap will enhance our understanding of the interplay between viral infection and fetal development and inform evidence-based interventions to mitigate adverse health outcomes in newborns.

1.4 Insights into Epigenetics and Epigenetic Mechanisms

The field of epigenetics, originating from the Greek prefix "epi-" meaning "beyond" along with "genetics" signifies its exploration of molecular mechanisms that control gene expression beyond the DNA sequence. The principal concept of "epigenetics" was initially introduced in 1942 by the

British biologist Conrad Waddington, who defined *epigenetics* as "the branch of biology which studies the causal interactions between genes and their products that result in the development of the phenotype" (Waddington, 2011). Epigenetics delineates a domain where gene expression undergoes heritable modulation without concomitant alterations in the underlying DNA sequence like mutations and translocations (Buenrostro et al., 2013; Harvey et al., 2018; Heard & Martienssen, 2014; Waddington, 2011, 2012; Zilbauer, 2014). The significance of epigenetic mechanisms is increasingly apparent as they navigate the interplay between the genetic code and environmental influences like diet, infections, and exposure to toxins like alcohol. Therefore, phenotypic variations, like disease onset, may be propelled by epigenetic mechanisms, notwithstanding the stability of cellular DNA sequences (Feil & Fraga, 2012). Commong conditions such as obesity and cardiovascular disease indicate that epigenetic mechanisms might be the elusive factor contributing to disease development through modifying gene expression patterns, potentially leading to adverse clinical consequences. This phenomenon is particularly pronounced during critical periods of human development, such as pregnancy and early infancy, when individuals are most vulnerable to epigenetic influences (Barua & Junaid, 2015; Zilbauer et al., 2016). Currently, three primary epigenetic mechanisms have been recognized that influence gene expression: DNA methylation, modifications to histones, and gene expression regulation of small, non-coding RNAs (e.g., micro RNAs). The DNA double helix coils around proteins known as histones, collectively forming a nucleosome. Nucleosomes serve as the fundamental units of chromatin, which ultimately constitute chromosomes. Chromatin can adopt either a condensed state called heterochromatin, housing mostly transcriptionally silent genes, or an open, active state known as euchromatin. For a gene to be activated, it must reside within euchromatin, where its DNA sequence is accessible to the cellular machinery responsible for initiating transcription. DNA methylation and histone modifications regulate the conformation of chromatin structure. In contrast, non-coding RNAs, such as microRNAs, regulate gene expression by binding to messenger RNAs, thereby preventing their translation into proteins (Bergman & Cedar, 2013; Doenecke, 2014; Fabian & Sonenberg, 2012; Vogel & Lassmann, 2014; Zentner & Henikoff, 2013; Zilbauer, 2014).

DNA methylation

DNA methylation is a valuable indicator for assessing specific epigenetic conditions and is catalyzed by the DNA methyltransferase (DNMT) family. In the human genome, there are five DNMT genes: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT1, DNMT3A, and DNMT3B possess functional methylation activities (Dong et al., 2001; Edwards et al., 2017; Lyko, 2018; Ren et al., 2018). In the methylation process, methyl groups are added from S-adenosine methionine to the 5' position of cytosine (5C), forming of 5-methylcytosine (5mC). Cytosine methylation modulates gene transcription by directly or indirectly interfering with the transcriptional machinery (Lewsey et al., 2016). In the mammalian genome, DNA methylation predominantly occurs at cytosine residues followed by guanine residues, a pattern known as CpG dinucleotides. These methylation patterns serves crucial roles in diverse biological processes characterized by distinct molecular functions (Bird, 1986). For instance, DNMT1 is integral to genomic imprinting, heterochromatin formation, and gene silencing. In the context of X chromosome inactivation, DNA methylation plays a pivotal role in long-term gene silencing, thereby contributing to cellular memory (Park & Kuroda, 2001). Apart from its methylation activity, DNMT1 is recognized for its involvement in the DNA damage response. Evidence from studies on B cell differentiation and induced DNA damage indicates that DNMT1 can function as a genome protector and an early responder to DNA double-stranded breaks (DSBs) in both B cells and colon cancer cell lines, regardless of its catalytic activity (Eads et al., 2002; Ha et al., 2011; Shaknovich et al., 2011). Furthermore, DNMT3a and DNMT3b are primarily essential for embryonic development and are instrumental in initiating de novo methylation across the genome (Arora & Tollefsbol, 2021; Hoang & Rui, 2020; Veland et al., 2019).

Post-translational modifications (PTM) of histone proteins

PTM of histone proteins represent another crucial epigenetic mechanism involving histone proteins comprising a nucleosome core, N-terminus domain, and C-terminus domain. Post-translational modifications such as acetylation, methylation, ubiquitylation, and phosphorylation occur on specific residues of the N-terminus tails, exerting significant influence over cellular processes, including transcription, repair, and replication (Corujo & Buschbeck, 2018; Izzo &

Schneider, 2016; Zhao & Garcia, 2015). These modifications alter the functionality of chromatin, resulting in either activation or repression depending on the specific residues undergoing modifications (Lawrence et al., 2016). For example, trimethylation of lysine four on histone H3 (H3K4me3) is associated with transcriptionally active gene promoters, while trimethylation of H3K9 and H3K27 occurs on transcriptionally suppressed gene promoters (Kouzarides, 2007; Ricketts et al., 2019). These histone variations are dynamically regulated by enzymes that add or remove covalent changes to the histone proteins, such as Histone acetyltransferases (HATs) and histone methyltransferases (HMTs) that add acetyl or methyl groups, respectively, while histone deacetylases (HDACs) and histone demethylases (HDMs) remove either acetyl or methyl groups (DesJarlais & Tummino, 2016; Woo et al., 2017). Alterations in histone patterns can lead to either transcriptional activation or silencing, with specific modifications such as H3K4me2, H3K4me3, H3R17me, H3K36me3, and H4R3me associated with activation, and H3K9me3, H3K27me3, H4K20me1, and H4K20me3 linked to transcriptional silencing (Arora & Tollefsbol, 2021; Hu et al., 2019).

Noncoding RNA (ncRNA)

ncRNA is increasingly recognized for its significant role in coordinating gene expression and cellular processes (Holoch & Moazed, 2015). One of the most extensively studied noncoding RNAs (ncRNAs) is microRNAs (miRNAs), consisting of short RNA sequences. Typically, miRNAs inhibit the translation of messenger RNA (mRNA) into protein by binding to a short complementary region, resulting in miRNA-induced silencing complex (miRISC) (Zilbauer et al., 2016). Research has indicated that epigenetic mechanisms, including DNA methylation, not only govern the transcription of protein-coding genes but also influence the expression of miRNAs. Conversely, miRNAs can modulate the expression of key epigenetic regulators, such as DNA methyltransferases and histone deacetylases. Thus, a dynamic regulatory network exists among various epigenetic pathways, collectively orchestrating gene expression profiles through transcriptional or post-transcriptional mechanisms (Moutinho & Esteller, 2017).

1.4.1 Pregnancy and Epigenetics

During gestation, epigenetic mechanisms play a pivotal role in regulating the intricate interplay between the fetus and the mother, impacting fetal development. These mechanisms respond dynamically to internal and external environmental factors, contributing significantly to fetal survival and health. The dysregulation of epigenetic pathways during gestation has been associated with a spectrum of defects in both the fetus and adolescents, highlighting the critical importance of epigenetic regulation in prenatal development (Gluckman et al., 2009; Gluckman et al., 2010; Gluckman et al., 2007; Pradhan et al., 2023; Solomons, 2009). Extensive epidemiological studies have identified various factors such as diet, obesity, stress, infection, and gestational diabetes during pregnancy as influencing normal fetal development. Recent discoveries have shed light on the role of epigenetic mechanisms during gestation, which are believed to contribute to various complications such as congenital heart defects (Barua & Junaid, 2015; Kleeman et al., 2022). Barua & Junaid, 2015 comprehensively explored environmental factors during pregnancy on health outcomes and potential diseases that may manifest later in life. Some of them are discussed below.

Maternal diet is raising concerns about the possibility of epigenetic modifications arising from excessive supplementation or inadequate nutrition during pregnancy, potentially causing substantial alterations in the epigenome of developing offspring. Throughout pregnancy, the presence of essential methyl donors such as folate, choline, serine, betaine, and methionine from dietary sources can impact the synthesis of S-adenosyl-methionine, thereby influencing DNA methylation and histone modifications within the fetal epigenome, ultimately leading to changes in gene expression. A study conducted in a mouse model demonstrated that supplementation with methyl donors before and during pregnancy induced epigenetic alterations, resulting in modifications to the phenotype towards a healthier and longer-lived direction; suggested research into optimal dietary supplements for the health and longevity of offspring should be thoroughly explored (Cooney et al., 2002; Waterland et al., 2007). Maternal obesity has been associated with increased adiposity, cardiovascular disease risk, and altered glucose metabolism in offspring that can potentially be inherited by future generations. Maternal high-fat diet exposure has been shown to induce changes in methylation patterns, resulting in reduced insulin sensitivity and increased weight gain in offspring mice across at least two generations (Dunn & Bale, 2009; Suter et al.,

2011). Research conducted in animal models has demonstrated that exposure to a high-fat diet during pregnancy disrupts lipid metabolism and inflammatory processes through epigenetic alterations and histone modifications, leading to non-alcoholic fatty liver disease (NAFLD) in the offspring (Strakovsky et al., 2014). Another concern regarding the epigenetic landscape arises from prenatal undernutrition, which has been observed to induce substantial modifications in the epigenetic profiles of offspring during intrauterine development. Research into the aftermath of the Dutch famine offers a unique opportunity to explore the effects of prenatal malnutrition on human development, revealing distinct methylation patterns that persist even six decades later (Heijmans et al., 2008; Tobi et al., 2009). Individuals who experienced Dutch Hunger Winter in 1944-45 during late or mid-gestation displayed reduced glucose tolerance, while those exposed earlier in gestation exhibited a lipid profile conducive to atherosclerosis, along with elevated fibrinogen levels and reduced plasma concentrations of factor VII. Furthermore, this group showed a higher body mass index (BMI) and appeared to have an increased risk of coronary heart disease (CHD) (Barua & Junaid, 2015; Roseboom et al., 2001).

Maternal obesity, including conditions such as gestational diabetes mellitus (GDM), can have detrimental effects on fetal programming during pregnancy, leading to long-lasting impacts on the phenotype and health of offspring like large-for-gestational-age infants, type 2 diabetes, and adiposity through specific DNA methylation patterns (Cedergren, 2006; del Rosario et al., 2014; Frederick et al., 2006; Godfrey et al., 2011; Sumathipala et al., 2012). Metabolic investigations on offspring exposed to a diabetic intrauterine environment have revealed impaired insulin secretory function in Pima Indians. This suggests that a deficiency in insulin secretion may underlie the heightened risk for type 2 diabetes (Gautier et al., 2001). GDM increases the risk of metabolic disorders, particularly those related to glucose-related disorders and cardiovascular disease, through alterations in epigenetic patterns in genes primarily implicated in metabolic pathways (Barua & Junaid, 2015; Clausen et al., 2009; Ruchat et al., 2013; West et al., 2013).

Maternal stress, such as maternal major depressive disorder (MDD) during pregnancy, may result in alterations in the developing brain that may endure into adulthood, posing a sustained risk for adult-onset chronic diseases and exerting profound effects on behavioral and cognitive-emotional functions (Salisbury et al., 2011; Sandman et al., 2011). Research indicates that infants whose mothers experienced depression during both pregnancy and infancy exhibit elevated

baseline cortisol levels compared to children not exposed to maternal depression or exposed only during infancy (Essex et al., 2002). This suggests that the prenatal environment significantly influences these adverse behavioral outcomes by affecting the development of the infant's brain, including the neuroendocrine system (Gudsnuk & Champagne, 2012; Lester et al., 2013). An epidemiological investigation has highlighted a significant correlation between maternal stressors and newborn birth weight. It has been suggested that fetal exposure to maternal corticosterone induced by stress may be one of the primary factors contributing to this association as indicated that third trimester depressed maternal mood has been linked to increased methylation levels of the human hippocampal glucocorticoid receptor gene NR3C1 in cord blood (Barua & Junaid, 2015; Hompes et al., 2013; Mulligan et al., 2012; Oberlander et al., 2008).

Maternal infection has been shown to induce alterations in the epigenome of offspring. Increasingly, prenatal exposure to infection is acknowledged to contribute significantly to the etiology of various brain disorders, including schizophrenia and bipolar disorder (Atladóttir et al., 2012; Brown & Derkits, 2010; Harvey & Boksa, 2012; Parboosing et al., 2013). Considering the significant role of epigenetic remodeling during early development, recent hypotheses suggest that epigenetic modifications may serve as a crucial molecular mechanism through which prenatal immune activation can influence changes in brain development and functions (Bohacek & Mansuy, 2015; Szyf, 2015). Research indicates that Maternal Immune Activation (MIA) can disturb cytokine and oxidative stress levels in the fetal brain, leading to epigenetic changes that impact long-term behavioral outcomes. For example, alterations in DNA methylation and histone modification, which affect genes like DISC1 associated with schizophrenia risk, have been observed in offspring exposed to MIA (Kleeman et al., 2022). Human enterovirus infection during pregnancy has been identified as a significant risk factor for developing Type-1 Diabetes (T1D) during childhood and adolescence by altering their epigenome (Allen et al., 2018; Dahlquist et al., 1995). Studies suggest that enterovirus alters the suppression of proinflammatory factors within pancreatic beta cells through miRNA regulation, thereby contributing to the increased likelihood of T1D in offspring later in life (Barchetta et al., 2021). Chorioamnionitis refers to the infection and inflammation of the chorion, amnion, and placenta. This condition has been linked to changes in neonatal immune responses and an increased risk of asthma during childhood. Elevated levels of maternal cytokines in the first trimester are associated with reduced methylation

of the MEG3 locus in neonatal mononuclear cells. MEG3 is a long non-coding RNA that plays a role in facilitating the transition from epithelial to mesenchymal cells and functions as a tumor suppressor, and it is plausible that could contribute to maternal inflammation-induced lung dysfunction (Bermick & Schaller, 2022; Fong et al., 2020; McCullough et al., 2017). Perinatal acquisition of human immunodeficiency virus (HIV) has lasting impacts on long-term health outcomes, such as cognitive impairments and metabolic syndrome. Blood samples from children who acquired HIV perinatally display distinct DNA methylation patterns compared to uninfected counterparts, including genes involved in pathways crucial for adaptive immunity, potentially playing a role in the enduring health effects observed in children with perinatally acquired HIV (Bermick & Schaller, 2022; Shiau et al., 2019). Congenital Zika virus infection is linked to severe microcephaly and adverse neurocognitive outcomes. Toddlers affected by congenital Zika virus infection and microcephaly exhibit distinct whole-blood DNA methylation patterns compared to unexposed children with typical head sizes. Specifically, there is hypomethylation observed in genes such as RABGAP1L, MX1, and ISG15. RABGAP1L is implicated in brain development, while MX1 and ISG15 are involved in host immunity against viruses and function to impede Zika virus replication. (Anderson et al., 2021; Bermick & Schaller, 2022; J. Chen et al., 2017). Folic acid, serving as a major dietary supplier of methyl groups, is poised to exert a direct impact on epigenetic mechanisms during typical prenatal development. Notably, recent research has unveiled a notable association between maternal folic acid intake and the methylation status of various genes in offspring, some of which are implicated in neurological functions and embryonic development. In the context of maternal infection, reduced folic acid availability may impinge on methylation-related epigenetic processes in the fetus, potentially contributing to infection-induced epigenetic programming (Joubert et al., 2016; Niculescu & Zeisel, 2002). The microbial composition of developing offspring may exhibit sensitivity to environmental shifts even during prenatal stages. A recent study in mice revealed that prenatal immune activation resembling viral infection can alter the offspring's gut microbiota composition. Remarkably, among the main metabolic byproducts of the gut microbiota are short-chain fatty acids, notably sodium butyrate. This compound possesses the ability to traverse the blood-brain barrier and influence the expression of various genes in brain, particularly those involved in neuronal activity regulation. Additionally, sodium butyrate can engage with the epigenetic machinery by inhibiting histone deacetylases (HDACs), thus serving as an effective epigenetic regulator (Davie, 2003; Hsiao et

al., 2013; Kratsman et al., 2016; Weber-Stadlbauer, 2017). Inflammatory cytokines also exert effects on the epigenetic machinery. It has been reported that increased expression of IL-17a in the fetus modulates histone deacetylase activity, while TNF-α causes histone acetylation activity (Zijlstra et al., 2012). Another study demonstrated that IL-6 induces increased biological activity of DNMT1, and the interplay between IL-6 and DNMT1 promotes cell migration, a crucial process for neurodevelopment in offspring (Basil et al., 2014; Hodge et al., 2007; Labouesse et al., 2015).

1.4.1.1 COVID-19 Impact on Pregnancy: Insights into Epigenetic Modulation

Studies have demonstrated that maternal infection with SARS-CoV-2 can impact the neonatal immune system despite the absence of vertical transmission of the virus. This impact is characterized by changes in the levels of inflammatory cytokines such as IL-6 in neonates and alterations in the responsiveness of neonatal immune cells. An elevation in the expression of IL-6 has been linked to cognitive deficits in offspring exposed to maternal immune activation (MIA) (Choi et al., 2016; Gee et al., 2021; Kleeman et al., 2022; Rudolph et al., 2018). MIA and systemic inflammation during maternal COVID-19 can alter epigenetic mechanisms. Furthermore, the escalated psychological strain experienced by mothers due to SARS-CoV-2 infection could also play a role in negatively influencing fetal reprogramming. Previous studies have shown that COVID-19 infection during pregnancy, like histological chorioamnionitis (HCA), correlates with distinct DNA methylation patterns corresponding to genes related to stress response (Fong et al., 2020; Hill et al., 2023; Kotsakis Ruehlmann et al., 2023). In umbilical cord blood cells of COVID-19-infected-mothers, 119 differentially methylated genes and canonical pathways related to the stress response, hepatotoxicity, nephrotoxicity, and cardiotoxicity were identified. This alteration could potentially impact child development (Urday et al., 2023). In contrast, Kocher et al. found no discernible epigenetic variances linked to maternal COVID-19 infection during pregnancy. They suggested that alterations in the neonatal epigenome are probably attributable to the broader environment shaped by the COVID-19 pandemic rather than specifically to the mother's COVID-19 infection status during pregnancy (Kleeman et al., 2022; Kocher et al., 2023).

1.5 Study Hypotheses and Research Objectives: A Closer Look

According to the literature review provided above, epigenetic mechanisms play a crucial role in regulating cell-specific functions and disease processes, and their disruption has been associated with various health conditions. Recent research suggests that infections during pregnancy can lead to changes in the epigenetic profiles of offspring, potentially affecting their long-term health. Moreover, the immune response elicited by SARS-CoV-2 in expectant mothers might have far-reaching effects across generations, exacerbating the impact of the pandemic on future populations. Thus, we hypothesize that COVID-19 during pregnancy can predispose the fetus to long-lasting health problems even in the absence of vertical transmission through epigenetic modulation. Our study aims to investigate how maternal infection with SARS-CoV-2 influences epigenetic modifications in offspring, mainly focusing on the risk of developing metabolic syndrome.

1.6 REACTOME

Reactome, an open-source pathway database, is a testament to the meticulous process of manual curation, peer review, and authorship by expert biologists. The pathway annotations, a result of collaborative efforts with Reactome editorial staff, are not just crafted but meticulously crafted. These annotations are then cross-referenced with numerous bioinformatics databases, ensuring the highest level of accuracy. The "Reactome_Epigenetic_Regulation_of_Gene_Expression" is a collection of 187 genes responsible for epigenetic regulation of gene expression, a product of such meticulousness, gathered by Bruce May and G Gopinathrao and reviewed by Lisa Matthews (MSigDB; REACTOME).

During cellular differentiation, cells specialize into specific types where gene expression patterns are established by polycomb complexes, namely PRC1 and PRC2. PRC2 methylates histones and DNA, creating initial repressive marks such as H3K27me3 and 5-methylcytosine in DNA. EZH2, a component of PRC2, specifically trimethylates lysine-27 of histone H3, resulting in H3K27me3, which serves as a binding site for the Polycomb subunit of PRC1. PRC1 then ubiquitinates histone H2A, maintaining repression. Epigenetic systems, including PRC2, also modulate gene expression through DNA methylation. DNA methyltransferases (DNMTs), namely DNMT1,

DNMT3A, and DNMT3B, facilitate the transfer of a methyl group from S-adenosylmethionine to the 5-position of cytosine in DNA. In contrast, TET1, TET2, TET3, and TDG oxidize the methyl group of 5-methylcytosine through TET enzymes, resulting in demethylation of DNA. Subsequently, TDG removes the oxidized product, which can take the form of either 5-formylcytosine or 5-carboxylcytosine. Ribosomal RNA (rRNA) genes are activated and deactivated in accordance with cellular metabolic needs. Positive regulation of rRNA expression involves chromatin modifications induced by activators such as ERCC6 (CSB), the B-WICH complex, and histone acetylases like KAT2B (PCAF). Negative regulation of rRNA expression entails chromatin modifications mediated by repressors such as the eNoSC complex, SIRT1, and the NoRC complex. WDR5 is a constituent of six histone methyltransferases and three histone acetyltransferases involved in the epigenetic control of gene expression. (more detailed information regarding this collection can be found at https://reactome.org) (Figure 1. 1) (Guarnaccia & Tansey, 2018; Jaenisch & Bird, 2003; REACTOME).

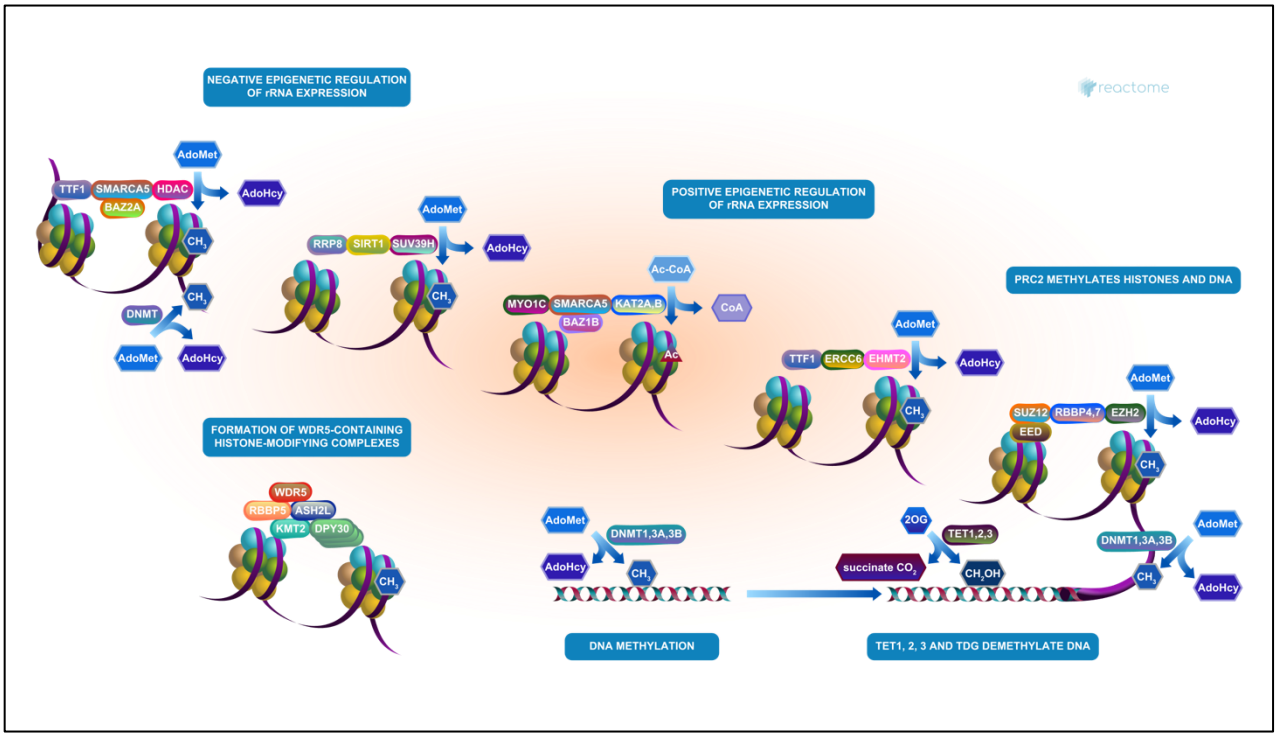

Figure 1. 1 Epigenetic Mechanisms Involved in Gene Expression Regulation According to Reactome Database (https://reactome.org)

1.7 How can Epigenetic Changes Impact the Health of the Fetus?

Metabolic syndrome is a complex condition characterized by a cluster of health issues, including obesity, insulin resistance, impaired glucose tolerance or diabetes, disturbances in lipid metabolism, and cardiovascular diseases (CVDs). This syndrome significantly contributes to morbidity and mortality globally. Recent studies propose that adverse conditions within the uterus, like maternal under and overnutrition, glucocorticoid exposure, and infection, can trigger fetal metabolic programming by negatively influencing the development of different cell types, tissues, and organ systems, culminating in obesity, insulin resistance, and metabolic syndrome in adult offspring. This phenomenon is primarily driven by fetal epigenetic programming, a pivotal mechanism in fetal metabolic programming which was first proposed by Barker and Hales in 1998 (Barker, 1998; Barrès & Zierath, 2016; Bošković & Rando, 2018; Grundy, 2005; Huypens et al., 2016; Sun et al., 2018; Tamashiro & Moran, 2010; Tang et al., 2016; Zhu & Cao, 2019).

An overview of the critical role of epigenetic programming in fetal metabolic programming of obesity and insulin resistence is provided by (Zhu et al., 2019) which is summarized here.

Epigenetic Programming of Obesity

There have been reports of global genome-wide epigenetic alterations in offspring associated with obesity or exposure to an adverse intrauterine environment. Specifically, gestational diabetes has been linked to widespread methylation changes in neonatal cord blood, adipose tissue, placenta, and peripheral blood leukocytes of offspring and adults (P. Chen et al., 2017; del Rosario et al., 2014; Haertle et al., 2017; Hjort et al., 2018; Kang et al., 2017; Reichetzeder et al., 2016; Weng et al., 2018). Additionally, DNA methylation alterations in the placenta or the cord blood have been associated with abnormal birth weight or BMI in childhood and adulthood (Hjort et al., 2018; Michels et al., 2011; Sharp et al., 2017; Simpkin et al., 2015; Van Dijk et al., 2018). Moreover, it

has been noted that circulating extracellular RNA, specifically ex-miR-122, correlates with regional adiposity in adults (Shah et al., 2017).

Epigenetic Programming of Insulin Resistance

Global genome-wide epigenetic changes have been documented to be linked with insulin resistance. Levels of DNA methylation collected from newborns have been associated with insulin sensitivity and BMI during childhood, such as methylation of retinoid X receptor-a (RXRA), TACSTD2 gene, and non-coding RNA nc886 (VTRNA2-1) (Godfrey et al., 2011; Relton et al., 2012; Van Dijk et al., 2018). A prospective cohort study conducted at Ewha Woman's University, MokDong Hospital (Seoul, Korea), spanning from 2003 to 2005, demonstrated that elevated methylation levels of pro-opiomelanocortin (POMC) in cord blood have been correlated with lower birthweight, higher triglycerides, and hyperinsulinemia in childhood (Yoo et al., 2014). Additionally, increased expression of insulin resistance-associated microRNA-15a and microRNA-15b has been observed in adult offspring of mothers with diabetes during pregnancy (Houshmand-Oeregaard et al., 2018). Furthermore, another study found an association between circulating extracellular RNA, particularly ex-miR-122, and insulin resistance in children and adults (Shah et al., 2017).

Epigenetic Programming of Cardiovascular Disease (CVD)

The risk factors for cardiovascular disease (CVD), such as lifestyle, genetic background, and suboptimal fetal development, are widely recognized. In response to adverse intrauterine conditions, the fetus adapts to ensure survival, resulting in immediate consequences such as low birth weight (LBW) and long-term effects, including an elevated susceptibility to CVD development in adulthood. This phenomenon, referred to as Developmental Origins of Health and Disease (DOHaD) or fetal programming of CVD, underscores the critical role of early-life conditions in shaping long-term health outcomes (Rodríguez-Rodríguez et al., 2018). Prenatal inflammation exposure (PIE) has garnered attention for over a decade. This inflammation can stem from three primarily sources, including the classic inflammatory pathway, often called "hot" inflammation, which is triggered by pathogens like viral infections (Calay & Hotamisligil, 2013). Prenatal inflammation exposure (PIE) can lead to CVDs in offspring named "PIE-programmed

CVDs". A noteworthy observation stems from a series of studies involving individuals born during the influenza pandemic of 1918; this demographic cohort exhibits a notably heightened prevalence of cardiovascular diseases (CVDs) (Cocoros et al., 2014; Mazumder et al., 2010; Myrskylä et al., 2013). Various fetal stress factors lead to hypertension and heart disease through common mechanisms, including deficient kidney development, blood vessel alterations, and heart abnormalities. These include reduced nephron count, vascular remodeling, and cardiac dysfunction (Rodríguez-Rodríguez et al., 2018). Deng et al. conducted initial investigations into the global DNA methylation levels of the renal cortex, revealing a significant increase in offspring exposed to prenatal inflammation (PIE). Furthermore, they observed elevated levels of DNA methyltransferase (DNMT) 1 and DNMT3B, enzymes responsible for DNA methylation, in renal tissue. Additionally, heightened levels of histone H3 acetylation at the ACE promoter were detected in the renal cortex of PIE-exposed offspring. This preliminary evidence underscores the pivotal role of epigenetic alterations in the development of cardiovascular diseases programmed by prenatal inflammation (Deng et al., 2018; Lyko, 2018; Wang et al., 2017; Wang et al., 2016; Weber-Stadlbauer, 2017).

1.8 Research Question

Does the expression of genes involved in epigenetic regulation show variation in umbilical cord blood cells from mothers infected with COVID-19 compared to controls? If so, which biological pathways are impacted in cell types showing changes in epigenetic regulation? To investigate these questions, we initially analyzed the differential expression of a Reactome set consisting of 187 genes associated with epigenetic mechanisms using the Linear Combination Test (LCT). Following this, to identify the biological pathways affected in cells displaying alterations in epigenetic patterns, we examined the differential expression of a Kyoto Encyclopedia of Genes and Genomes (KEGG) collection. Our aim is to pinpoint potential metabolic disorders stemming from Maternal Immune Activation induced by SARS-CoV-2.

1.9 Molecular Signatures Database (MSigDB)

The Human Molecular Signatures Database (MSigDB) contains 34,550 gene sets categorized into nine main collections, each with several subcollections (H; hallmark gene sets, C1; positional

gene sets, C2; curated gene sets, C3; regulatory target gene sets, C4; computational gene sets, C5; ontology gene sets, C6; oncogenic signature gene sets, C7; immunologic signature gene sets, C8; cell type signature gene sets) (www.gsea-msigdb.org).

The gene sets within this collection are sourced from diverse outlets such as online pathway databases, biomedical literature, and contributions by individual experts in the respective domains. Each gene set page specifies its origin. Specifically, the C2 collection is subdivided into two main categories: Chemical and genetic perturbations (CGP) and Canonical pathways (CP). The pathway gene sets are curated from a variety of online databases, including BioCarta, KEGG MEDICUS, Pathway Interaction Database, Reactome, SigmaAldrich, Signaling Gateway, SuperArray SABiosciences, WikiPathways, and KEGG Legacy Sets. The Canonical Pathways gene sets are derived from the KEGG pathway database and include 186 gene sets attributed to seven collections of KEGG pathway.

1.10 KEGG: Kyoto Encyclopedia of Genes and Genomes

KEGG serves as a valuable database resource for comprehending biological systems broad functions ranging from cellular processes to entire ecosystems. It accomplishes this by integrating molecular-level data, particularly large-scale datasets generated through genome sequencing and high-throughput experimental technologies. The KEGG model comprises sixteen integrated databases, categorized into systems information, genomic information, chemical information, and health information. The KEGG pathway is within the systems information category (https://www.genome.jp/kegg/).

KEGG PATHWAY is a collection of manually drawn pathway maps, separated into seven clusters, representing our knowledge of the molecular interaction, reaction, and relation networks for various biological processes, cellular functions, and organismal systems.

1. Metabolism
   Global/overview maps
   Carbohydrate metabolism
   Energy metabolism
   Lipid metabolism
   Nucleotide metabolism

Amino acid metabolism
Other amino acid metabolism
Glycan biosynthesis and metabolism
Metabolism of Cofactor/vitamin
Metabolism of Terpenoid/ polyketides (PK)
Biosynthesis of another secondary metabolite
Xenobiotics biodegradation and metabolism
Chemical structure transformation maps

2. Genetic Information processing
3. Environmental Information Processing
4. Cellular Processes
5. Organismal Systems
6. Human Disease
7. Drug Development

Chapter 2: Dataset and Methodology

To demonstrate epigenetic and metabolic pathways alteration in the neonates born to mothers infected with COVID-19 without vertical transmission, we used a gene expression dataset accessed on the Gene Expression Omnibus database (GSE 165193). Droplet-based single-cell RNA-sequencing (SC-RNA Seq) and T-cell receptor sequencing were performed on cord blood mononuclear cells (CBMCs) from three-term gestation infants (>37 weeks) of infected mothers with SARS-CoV-2 having mild symptoms (cases) according to NIH, 2020 guideline, and non-exposed three infants during the same epoch (controls). In this study, none of the three infants born to mothers with SARS-CoV-2 tested positive for the virus postnatally as exhibited by detectable SARS-CoV-2 messenger RNA (mRNA) in the placenta or experienced any neonatal morbidity. All mothers with COVID-19 in the third trimester were classified as having mild disease and did not require respiratory support. Maternal comorbidities, including well-controlled thyroid dysfunction, obesity, or gestational diabetes, were matched between cases and controls to the extent possible (Table 2. 1). The dataset includes 25,970 cells with high-quality single-cell transcriptomes, 14,748 cells from cases, and 11,222 cells from controls (Table 2. 2). The visualization of the cell population composition was achieved by the utilization of uniform manifold approximation and projection (UMAP). Additionally, the identification of cell types was determined by analyzing cluster-specific canonical marker genes. CD14+ monocytes were stratified into five clusters, while CD16+ monocytes were grouped into one cluster. Additionally, two distinct clusters of CB NK cells were identified. One NK cell population (cluster 1) exhibited elevated expression of GZMB, indicative of CD56dim phenotype, whereas the second NK cell population (cluster 2) expressed IL7R and XCL1, suggesting a CD56bright phenotype. B cells were classified into three clusters, and three clusters of T cells were also delineated. Cluster 1 of T cells corresponded to cytotoxic (CD8+) T cells, while Clusters 2 and 3 represented helper T cells. Increased expression of CCR7 in T cell Cluster 2 suggests the presence of either naive T cells or central memory T cells. In contrast, elevated expression of CTSW and KLRB1 in Cluster 3 indicates the inclusion of effector and memory T cells. No significant variations in cell cluster composition were reported by the authors between cases and controls (Table 2. 3) (Figure 2. 1) (Matute et al., 2022).

In this study, to investigate transcriptional signatures in fetal immune cells linked to maternal SARS-CoV-2 infection, Matute et al. conducted differential gene expression (DGE) analysis within 18 cell types, comparing cases and controls. Genes with a false discovery rate (FDR) < 5% were deemed statistically significant. Their study revealed alterations in the expression of hundreds of genes across nearly all cell types. Subsequently, they employed gene ontology (GO) analysis to categorize genes significantly affected by maternal SARS-CoV-2 infection based on DGE. However, single-gene analysis encounters several limitations, which we address below.

Table 2. 1 Clinical Characteristics of Cases and Controls (Matute et al., 2022)

Subjects	*Onset of Symptoms (GA)*	*Delivery (GA)/ Mode*	*Dyas between the onset of symptoms and birth*	*SARS-CoV-2 PCR (GA)*	*Maternal symptoms at test*	*Sex at birth*	*Maternal comorbidities*	*Placental Viral load by RT-PCR*	*24h nasopharyngeal viral load by RT-PCR*
Control 1	NA	40 / VD	NA	39.9	NA	M		1	NA
Control 2	NA	39w 4d / CS	NA	38.9	NA	F	BMI > 30, Thyroid Disease	1	NA
Control 3	NA	38w 6d / CS	NA	38.4	NA	F	BMI > 30	ND	NA
Case 1	30w 3d	39w 7d / VD	66	30w 7d	Fever/Chills, Nasal Congestion, Loss of Taste/ Smell, Sore Throat, Night sweats	M	Thyroid Disease	1	Negative
Case 2	34w 4d	40w 1d / CS	40	35w 4d	Cough, Fever/Chills, Myalgias, Headache, Chest Discomfort	M	Diabetes/ GDM, BMI > 30, Thyroid Disease	1	Negative
Case 3	39w 0d	40w / VD	7	39w 4d	Cough, Fever/Chills	F	BMI > 30	1	Negative

GA gestational age, VD vaginal delivery, CS caesarean section, ND not done, NA not applicable,

I: infected or detected.

Table 2. 2 Dataset Overview

Dataset Summary	***Counts***
Number of Cases	3
Number of Controls	3
Total Samples of Cases	14,748
Total Samples of Controls	11,222
Total Samples	25,970
Number of genes	36,601

Table 2. 3 Umbilical Cord Blood Cell Type Distribution

Cell Type	***Total***		***Cases***		***Controls***	
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)
B Cells 1	2,588	9.97	1,226	8.31	1,362	12.14
B Cells 2	1,821	7.01	909	6.16	912	8.13
B Cells 3	922	3.55	463	3.14	459	4.09
B Cells (Total)	5,331	20.53	2,598	17.61	2,733	24.36
CD14Monocytes1	2,749	10.59	1,608	10.9	1,141	10.17
CD14 Monocytes 2	2,124	8.18	1,093	7.41	1,031	9.19
CD14 Monocytes 3	1,821	7.01	958	6.5	863	7.69
CD14 Monocytes 4	1,493	5.75	783	5.31	710	6.33
CD14 Monocytes 5	980	3.77	569	3.86	411	3.66
CD14 Monocytes (Total)	9,167	35.3	5,011	33.98	4,156	37.04
CD16 Monocytes	480	1.85	325	2.2	155	1.38
T Cells 1	1,478	5.69	1,017	6.9	461	4.11
T Cells 2	3,881	14.94	2,500	16.95	1,381	12.31
T Cells 3	720	2.77	484	3.28	236	2.1
T Cells (Total)	6,079	23.4	4,001	27.13	2,170	18.52
Conventional DCs	397	1.53	220	1.49	177	1.58
HSPCs	151	0.58	83	0.56	68	0.61
Megakaryocytes	771	2.97	520	3.53	251	2.24
NK Cells 1	3,110	11.98	1,744	11.83	1,366	12.17
NK Cells 2	302	1.16	156	1.06	146	1.3
NK Cells (Total)	3,412	13.14	1,900	12.89	1,512	13.47
Plasmacytoid DCs	182	0.7	90	0.61	92	0.82

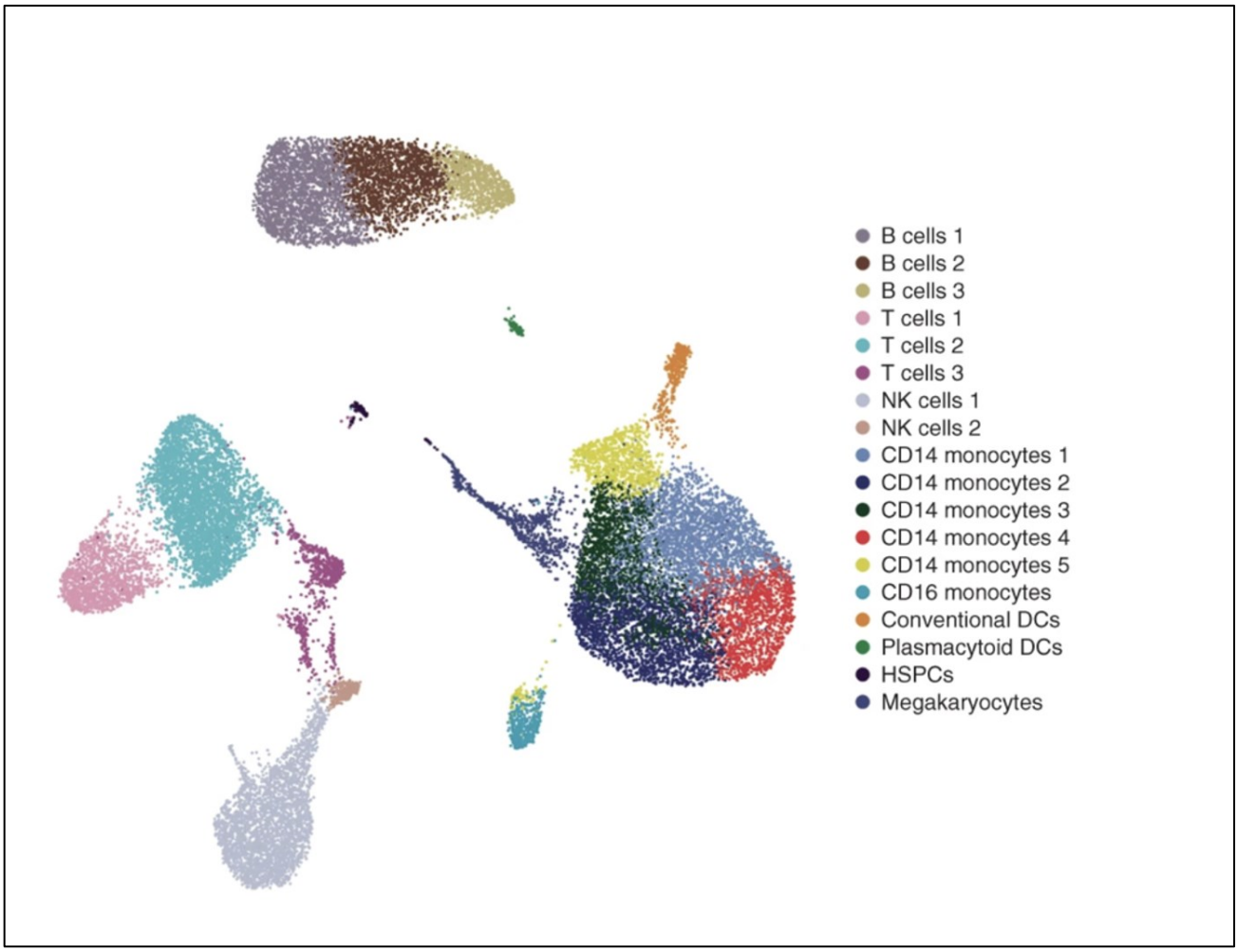

Figure 2. 1 Visualization of Cell Composition of CBMCs Obtained by single-cell RNA sequencing (scRNAseq). *Uniform Manifold Approximation and Projection (UMAP) of all Cases and Control CBMCs, with Cell Populations Labeled by Color. N = 3 Samples Per Group (Matute et al., 2022).*

2.1 Why Gene Set Level Analysis?

The initial method for analyzing gene expression data involved single-gene analysis, wherein the expression levels of individual genes in case and control samples were compared by fold change or t-test. A primary method consists of assessing the fold change between the control and experimental groups. Generally, a difference is deemed significant if it reaches at least two- to three-fold. Fold change is not considered a statistical test and doesn't provide a quantifiable measure of confidence in categorizing genes as differentially expressed. This method is susceptible to bias since genes with low intensity (meaning they are expressed at low levels) may appear differentially expressed more frequently than genes with high intensity. This happens because the fold-change values (the degree of change in expression between two conditions) for low-intensity genes tend to have a more significant variation than those for high-intensity genes. As a result, these low-intensity genes may show more significant fluctuations in expression levels, leading to the incorrect identification of differential expression. T-test is also used to compare the means of gene expression between two groups, and the hypothesis being tested states that there is no variation in expression levels between the conditions. This test essentially functions as a fold-change assessment because the global t-test ranks genes similarly to fold change, without adjusting for individual gene variability. Consequently, it may encounter the same biases as a fold-change test if the error variance does not remain constant across all genes. To address the challenge of erroneous positive findings stemming from numerous comparisons, corrective measures can be implemented, including the application of familywise error rate (FWER) controls such as Bonferroni correction and false discovery rate control (Cui & Churchill, 2003; Draghici, 2002; Maleki et al., 2020; Matute et al., 2022; Quackenbush, 2001). In their investigation, Matute et al. (2022) employed fold change as a metric to evaluate variances in gene expression between COVID-19 infected mothers and control groups. Genes exhibiting adjusted p-values below a predetermined threshold (0.05) were recognized as differentially expressed. Subsequently, these genes were subjected to biological interpretation.

Traditional strategies of single-gene analysis, while valuable, often overlook biological phenomena like metabolic pathways, transcriptional programs, and stress responses, which are distributed among numerous genes and may be subtle at the level of individual gene expression (Subramanian et al., 2005). Multi-functional genes, which are genes involved in multiple

biological processes, are frequently observed across diverse organisms; thus, a study based on individual genes may result in erroneous or unequivocal findings. High-throughput gene expression studies like single-cell RNA seq (scRNAsq) necessitate adjustments for multiple comparisons due to the large number of genes and cells analyzed. However, such adjustments may result in numerous false negatives, as they may detect very few or even no genes as differentially expressed. Also, the primary challenges inherent in the Individual Gene Analysis (IGA) process originate from using a predefined threshold value. The ultimate biological conclusions of IGA are significantly influenced by the selected threshold, typically established arbitrarily. Pan et al. suggest employing a variety of threshold conditions as it allows for evaluating the sensitivity of biological conclusions to threshold selection and assessing the overall robustness of conclusions (Pan et al., 2005). In addition, one notable concern regarding IGA is the erroneous assumption of independent sampling of genes, leading to an elevated occurrence of false positive predictions (Nam & Kim, 2008). Cellular processes frequently exhibit alterations in the expression patterns of gene clusters that possess shared biological activities. An overall alteration in a cluster of these genes possesses more biological reliability and interpretability compared to an alteration in an individual gene. The single-gene strategy needs to consider this information. Publicly available online databases such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) offer valuable insights into some of these gene sets made publicly available by the MSigDB team (Kanehisa et al., 2016). Despite the advancements in high-throughput technology that enable the simultaneous monitoring of gene expression for thousands of genes in a single experiment, researchers are faced with the issue of managing large dimensional data. Utilizing groupings of biologically related genes is a highly intuitive and biologically pertinent strategy for reducing dimensionality, computational challenges, and noise in high-throughput gene expression research (Berrar et al., 2003; Friedman, 1997). When the measured values for a single gene vary only slightly across multiple treatments, it becomes challenging to distinguish the actual difference in gene expression from the variation caused by biological differences in the samples. In contrast, gene set analysis has the potential to identify minor yet consistent alterations in the expression patterns of genes belonging to a specific gene set (Subramanian et al., 2005). The utilization of a single-gene technique has the potential to identify a substantial number of genes. Interpreting a lengthy inventory of genes that exhibit differential expression is a laborious undertaking that is susceptible to investigator bias toward a specific hypothesis of interest (Maleki et al., 2020).

2.1.1 Gene Set Analysis Methods

Gene set analysis, or enrichment analysis, aims to address these limitations and extract meaningful insights from gene expression data. Its primary objective is to identify whether there is enrichment or depletion in the expression levels of a specific set of genes, known as a gene set. Various Gene Set Analysis (GSA) methods have been developed to identify gene sets associated with specific phenotypes, each with distinct methodological assumptions and exhibiting diverse requirements, strengths, and weaknesses (Huang et al., 2009; Mitrea et al., 2013). These methods can be broadly categorized as "self-contained" or "competitive" (Goeman & Bühlmann, 2007). Competitive methods compare gene associations within a set to those outside the set to determine if genes within a specific set are more associated with a phenotype. Examples of competitive GSA methods used in gene expression studies include Gene Set Enrichment Analysis (GSEA), Significance Analysis of Function and Expression (SAFE), random set methods, and GSA. In contrast, self-contained methods assess the relationship between a phenotype and the gene set of interest without considering other genes outside that set. Examples of self-contained GSA methods include the Global test, ANCOVA, SAM-GS, and LCT (Figure 2. 2) (Wang et al., 2011; Tian et al., 2005).

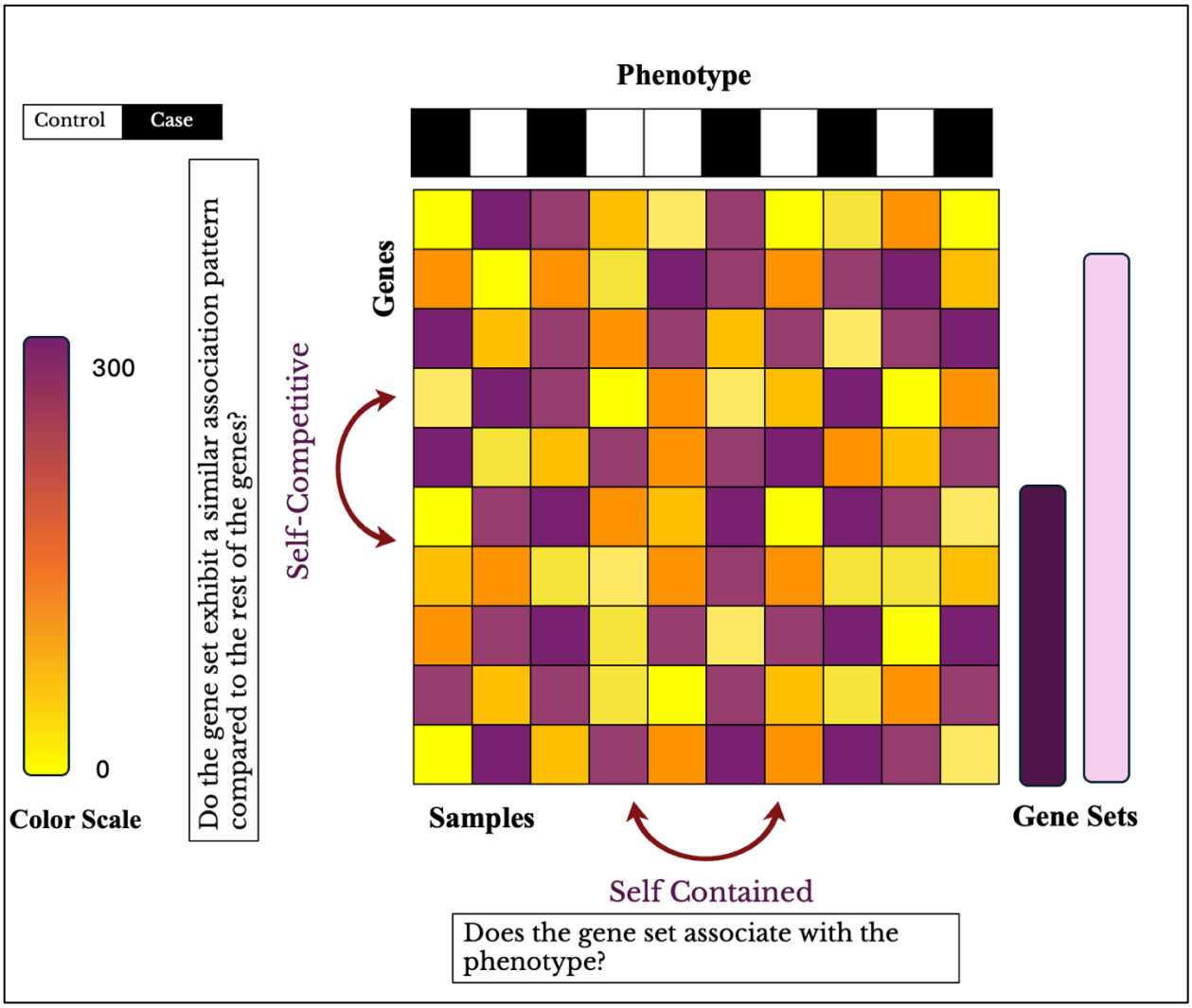

Figure 2. 2 Outline of Self-Contained and Self-Competitive Gene Set Analysis Methodologies (Tian et al., 2005). *Firstly, the expression level of genes across all samples is assessed by gene expression analysis tools like single-cell RNAseq (scRNAseq). Secondly different testing methodologies are applied to discern gene sets or pathways of statistical significance.*

2.2 Methodology: Linear Combination Test (LCT)

To identify differentially expressed gene sets stratified by cell type, considering case vs. control status in the context of maternal COVID-19, we performed LCT.

We chose LCT to effectively address the high dimensionality problem and the correlation of expression measurements often present across sets of genes and/or biological pathways via a shrinkage covariance matrix estimation approach. It is superior in terms of power and computational efficiency when compared to other gene-set analysis methods in both simulations and real data analysis (Wang et al., 2011).

To grasp the functionality of LCT, consider a gene expression dataset that involves a total of n individuals. Within this group, n_1 individuals are categorized as cases, while n_2 are categorized as controls. We are interested in identifying differentially expressed gene sets considering cases and controls. The null hypothesis posits no distinction in the expression of a predefined group of genes, denoted as ($X_1, \ldots, X_K$), between two distinct phenotypic clusters. One way to rephrase this hypothesis from a multivariate angle to a univariate standpoint is by stating H_0: There is no association between the phenotype and any linear combination of ($X_1, \ldots, X_K$).

Now, consider the function $Z(\boldsymbol{\beta})$, which can be represented as a linear combination of ($X_1, \ldots, X_K$), $Z(\boldsymbol{\beta}) = \beta_1 X_1 + \ldots + \beta_K X_K$. The link between the $Z(\boldsymbol{\beta})$ and the phenotype can be assessed using a vector $\boldsymbol{\beta}$ of coefficients through the subsequent univariate model. The equation $Z_{ij}(\boldsymbol{\beta}) = \mu_i + e_{ij}$ is utilized, where μ_1 and μ_2 denote the average gene expressions for cases and controls correspondingly. Additionally, e_{ij} follows a normal distribution with an average of 0 and a variance of σ^2, $e_{ij} \sim N(0, \sigma^2)$. Here, j pertains to individuals $1, \ldots, n_i$ within groups $i = 1, 2$. When scrutinizing the null hypothesis (H_0), the customary approach involves assessing the most impactful linear combination derived from the variable set ($X_1, \ldots, X_K$). This specific linear combination is determined by its exceptional statistical significance compared to all other potential linear combinations. The coefficients of the most significant linear combination are represented by:

$$\beta^* = \operatorname{argmax} T^2(\beta)$$

As the square root of the two-sample t-test, we have:

$$T^2(\beta) = \frac{\bar{Z}_1 - \bar{Z}_2}{S^2_{Z_1 Z_2}(\frac{1}{n_1} + \frac{1}{n_2})}$$

Where:

$\bar{Z}_1 = \beta^T \bar{X}_1$ $\bar{Z}_2 = \beta^T \bar{X}_2$ $S^2_{Z_1 Z_2} = \beta^T \widehat{\Omega} \beta$

$\bar{X}_1$: K sample average of gene expressions within cases

$\bar{X}_2$: K sample average of gene expressions within controls

$\widehat{\Omega}$: pooled covariance matrix over the two groups of the phenotypes

Therefore, the test statistic becomes:

$$T^2(\beta) = \frac{\beta^T(\bar{X}_1 - \bar{X}_2)(\bar{X}_1 - \bar{X}_2)^T\beta}{\beta^T(\frac{1}{n_1} + \frac{1}{n_2})\widehat{\Omega}\beta}$$

The solution to this optimization problem can be expressed as:

$$\beta^* = \text{argmax}_\beta \frac{\beta^T A \beta}{\beta^T B \beta}$$

A and B are the corresponding matrices in the numerator and denominator of the test statistic. The solution to this optimization problem can be calculated as the maximal eigenvector of AB^{-1} and $T^2(\beta^*)$ is the corresponding eigenvalue. To tackle problems with small sample size and large gene sets, LCT uses a shrinkage covariance matrix. The computational cost is significantly reduced by the orthogonal transformation of the original gene expression measurement, obtained using eigenvalue decomposition of the shrinkage covariance matrix.

$$\Omega^* = UDU^T$$

$$T^2(\gamma) = \frac{\gamma^T(\bar{Y}_1 - \bar{Y}_2)(\bar{Y}_1 - \bar{Y}_2)^T\gamma}{\gamma^T\gamma}$$

where:

$\gamma = D^{1/2}U^T\beta$

$\bar{Y}_1$: K sample averages within cases

$\bar{Y}_2$: K sample averages within controls

The coefficients of the most-significant combination are given by the mean vector difference between the two groups:

$$\gamma^* \propto (\bar{Y}_1 - \bar{Y}_2)$$

This LC test statistic is, therefore, proportional to the L_2-norm of the mean-vector difference between the two groups, after the orthogonal transformation:

$$T^2(\gamma^*) = c \parallel \bar{Y}_2 - \bar{Y}_2 \parallel$$

The constant *c* can be ignored in the permutation test.

A simulation study conducted by Wang et al. demonstrated that the LCT testing approach exhibits greater computational efficiency compared to the top two GSA methods, including modified Hotelling's T2 (Tsai & Chen, 2009) and SAM-GS (Dinu et al., 2007), while approximating its superior testing power. The core computational feature of LCT lies in its reliance on orthogonally-transformed features, necessitating the computation of the eigenvalue decomposition of the shrinkage pooled covariance matrix only once rather than for each permutation (Wang et al., 2011).

2.3 Analysis

2.3.1 Step 1

LCT was performed to demonstrate differential expression of "Reactome_epigenetic_regulation_of_gene_expression" in umbilical cord blood cells of neonates born to mothers infected with COVID-19 in the absence of vertical transmission compared to controls stratified by cell types. Cell types with p_value <= 0.01 are considered significant. Hematopoietic stem and progenitor cells (HSPCs), lacking distinct clusters, are excluded from subsequent analyses (Figure 2. 3).

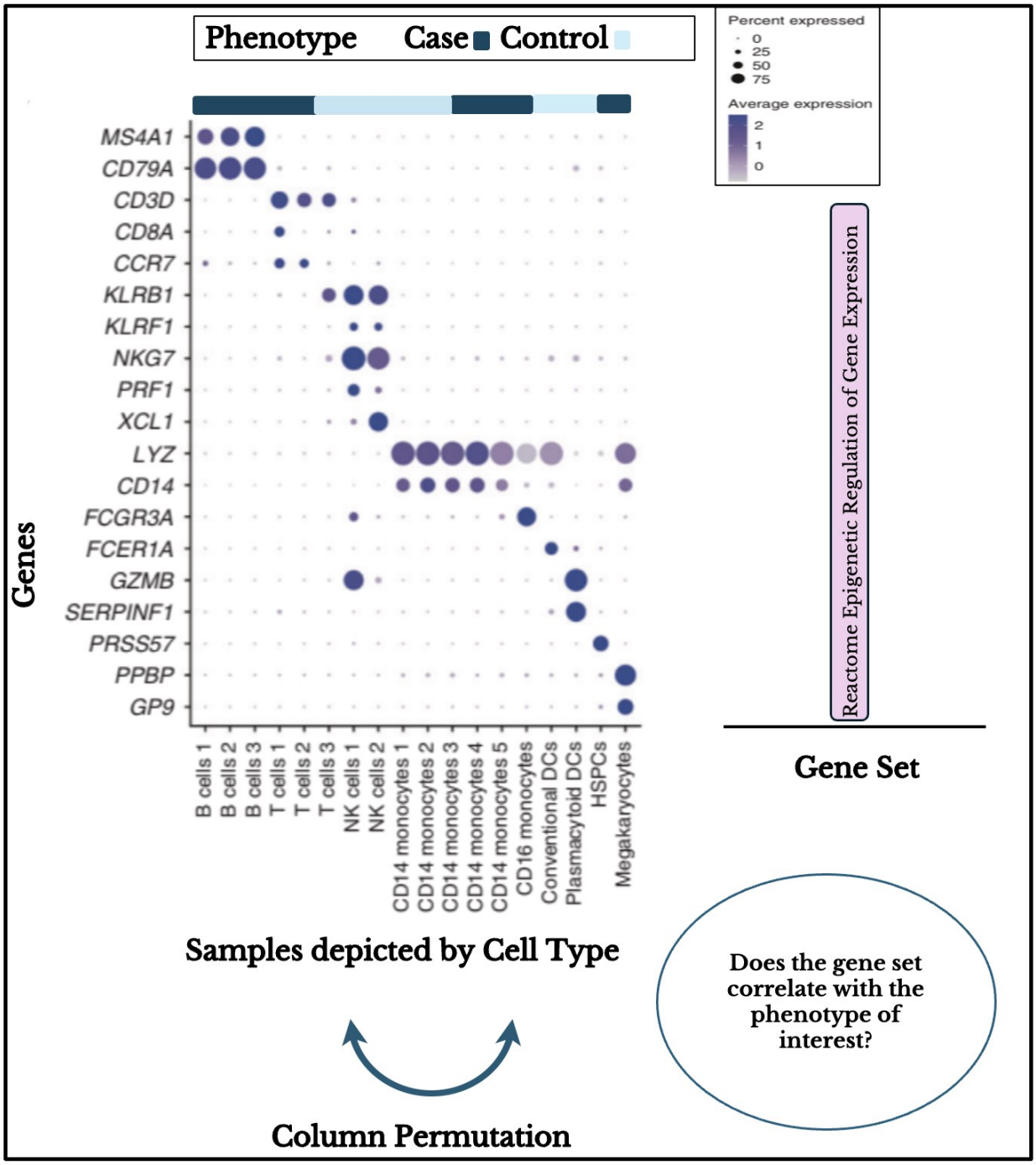

Figure 2. 3 Outline of Step 1 of Analysis. *Expression level of genes according to the cell type is assessed by single-cell RNA sequencing (scRNAseq). LCT considers column permutation to answer this question; Does the Reactome Epigenetic Regulation of Gene Expression collection correlate with the phenotype (COVID-19)*

considering cell type? Or does this collection differentially expressed given cases and controls stratified by cell type? This gene expression figure is extracted from the dataset GSE: 165193 and phenotype and gene set labels are depicted just for explanation purposes, Case: newborns of mothers infected with Covid-19. Control: newborns of healthy mothers (Matute et al., 2022).

2.3.2 Step 2

Next, to further assess which KEGG_LEGACY pathways are differentially expressed due to maternal COVID-19, we performed pairwise comparisons based on "Reactome_epigenetic_regulation_of_gene_expression" collection results only in infected samples. In detail, considering the KEGG collection, we performed LCT comparing B cell 2 with B cell 1 and 3 in maternal COVID-19 samples, thus finding which KEGG gene sets are differentially expressed in B cell 1 and 3 in cases (which had epigenetic signatures) compared to B cell 2 (which did not have any epigenetic alteration, as control for those cell types). Similarly, LCT was done regarding CD14 monocyte 4 as a control for other CD14 monocyte clusters, T cell 1 as a control for T cell 2 and 3, and NK cell 2 as a control for NK cell 1, in maternal COVID-19 samples. As such, we had 9 comparisons in total: B cell 2 vs B cell 1, B cell 2 vs B cell 3, CD14 monocyte 4 vs. CD14 monocyte 1, CD14 monocyte 4 vs. CD14 monocyte 2, CD14 monocyte 4 vs. CD14 monocyte 3, CD14 monocyte 4 vs. CD14 monocyte 5, NK cells 1 vs. NK cells 2, T cells 1 vs. T cells 2, T cells 1 vs. T cells 3 (Table 2. 4).

Table 2. 4 Representation of Step 2 Comparative Analysis of KEGG Collection Across Nine Comparisons. *Each row represents a KEGG gene set. Each column represents a pairwise comparison stratified by cell type. [p_1 … p_9] indicate the p-values obtained from LCT, assessing the differential expression of gene sets between the specified groups. C p_value, combined p-value, is calculated by Fisher's method.*

KEGG	***Comparisons Stratified by Cell Type***									
	Comparison Number									
	1	2	3	4	5	6	7	8	9	
	B cells		CD 14 Monocyte				NK Cells	T Cells		*C P_value
Gene set number	1 vs 2	3 vs 2	1 vs 4	2 vs 4	3 vs 4	5 vs 4	2 vs 1	2 vs 1	3 vs 1	
1	p_1	p_2	p_3	p_4	p_5	p_6	p_7	p_8	p_9	C p_value1
2	p_1	p_2	p_3	p_4	p_5	p_6	p_7	p_8	p_9	C p_value2
…	p_1	p_2	p_3	p_4	p_5	p_6	p_7	p_8	p_9	C p_value…
186	p_1	p_2	p_3	p_4	p_5	p_6	p_7	p_8	p_9	C p_value186

2.3.3 Step 3

Fisher's method, or Fisher's combined probability test combined the results of nine independent comparisons (nine p_values for each KEGG gene sets) into a single overall p-value (Fisher et al., 1928). This combined p-value indicates the strength of evidence against the null hypothesis when considering all nine LCT results for each KEGG gene set together.

As a reminder, the null hypothesis is stated as follows: H_0 = none of the given p values (p_1…, p_9) are associated with phenotype. Put simply, when considering each KEGG gene set, none of the comparisons revealed any significant differences.

At first, the formula below combines these p_values into a test statistic:

$$T = -2 \sum_{i=1}^{n} \log(p_i) \sim x^2(2n)$$

T is the test statistic following a chi-squared distribution.

p_i is the p-value obtained from the i-th independent test.

n = 9

Then, the combined p-value is calculated as the right-tail probability $p_{x^{2(2n)}}$ (T > t), where t is the observed T value.

Finally, we created a jitter plot of (X[g], Y[g]) with color grouping:

X[g] = Number of Comparisons where Significant (p_value <0.01)

Y[g]= Combined Significance over all Comparisons

2.4 Software and Packages

Before conducting the analysis, it was necessary to address the zero inflation in the gene expression data. To handle this issue, the 'jitter' function from the base R package was employed for 14,000 genes with zero expression values (Team, 2009). This function transforms zero expressions into small values that are in proximity to zero. Essentially, 'jitter' adds a slight, random deviation to the zero values, making the data more suitable for analysis. This preprocessing step helps mitigate the impact of excessive zero values, enabling more accurate and meaningful results

in subsequent analyses (Chambers, 1983; Chambers, 1992). All statistical computations were conducted utilizing R software (version 4.3.2). The analysis made use of the following R packages:

The data.table package facilitated rapid aggregation of extensive datasets.

The corpcor package enabled efficient estimation of covariance and (partial) correlation.

The qvalue package was utilized for estimating Q-values.

Chapter 3: Results

3.1 Step 1

Our findings revealed that in specific cell types of umbilical cord blood samples from mothers infected with COVID-19, compared to controls, there was differential expression of the "Reactome_Epigenetic_Regulation_of_Gene_Expression". This suggests that genes involved in epigenetic mechanisms show differential expression in specific cell types of umbilical cord blood from COVID-19-infected-mothers, indicating their susceptibility to epigenetic modulation. This susceptibility may contribute to the complex outcomes associated with alterations in epigenetic mechanisms like neurodevelopmental disease and metabolic syndrome as discussed previously. Another intriguing finding was the presence of heterogeneity within cell types, such as B cells. Specifically distinct results were observed within a particular cluster of B cells, referred to as B cell 2, compared to B cell 1 and B cell 3. This variability was also evident within other cell types, including CD14 monocytes, NK cells, and T cells (Table 3. 1).

Table 3. 1 LCT results of Reactome Collection Comparing Cases vs Controls Stratified by Cell Type

Cell Types	***p_value***	***q_value***
B Cells 1	0	0
B Cells 2	0.31	0.41
B Cells 3	0	0
CD14 Monocytes 1	0	0
CD14 Monocytes 2	0.002	0.003
CD14 Monocytes 3	0.01	0.02
CD14 Monocytes 4	0.41	0.45
CD14 Monocytes 5	0	0
NK Cells 1	0	0
NK Cells 2	0.49	0.49
zT Cells 1	0.36	0.43
T Cells 2	0	0
T Cells 3	0	0
HSPCs	0.005	0.009
CD16 Monocytes	0.02	0.04
Megakaryocytes	0.64	0.83
Conventional DCs	0.74	0.83
Plasmacytoid DCs	0.86	0.86

3.2 Step 2 and 3

For each gene set in the KEGG collection, we obtained nine p-values from nine pairwise comparisons (Table 3. 2). To consolidate p-values into a single summary statistic, we used Fisher's method function to obtain combined p_values and created a jitter plot (Figure 3. 1). The dots represent the 186 gene sets, Y[g] is an indicator of combined p values, and X[g] is the number of comparisons with p_value < 0.01. We designated the color of dots as the comparison number (1 to 9) for which the LCT p-value is minimum. Gene sets depicted on the left side of the figure exhibit differential expression in specific clusters regarding pairwise comparison. Conversely, gene sets on the right side of the figure demonstrate differential expression across almost all comparisons. This classification enables prioritization of further investigation and interpretation of the results into unique and biologically relevant gene sets while deprioritizing those with consistent differential expression. Limiting results to at most three comparisons, we have ten differential expressed gene sets, which are summarized in Table 3. 3.

Table 3. 2 LCT Results of Nine Comparisons regarding KEGG Gene Sets with Minimum Combined p_values.

KEGG	Comparisons Stratified by Cell Type									T Statistic	*C p_value
	Comparison Number										
	1	2	3	4	5	6	7	8	9		
Gene Sets	*B cells*		*CD 14 Monocyte Cells*				*NK*	*T cells*			
	1 vs 2	3 vs 2	1 vs 4	2 vs 4	3 vs 4	5 vs 4	2 vs 1	2 vs 1	3 vs 1		
Taurine_and_Hypotaurine_Metabolism	0.03	0.42	0.33	0.09	0.24	**0**	0.03	0.02	0.06	56.52	7.27E-06
Non_Homologous_End_Joining	0.04	**0**	0.30	0.02	0.13	0.02	0.15	0.04	**0.004**	66.41	1.81E-07
Glycosphingolipid_Biosynthesis_Globo_Series	0.61	0.42	0.09	**0**	**0.005**	0.03	0.05	0.16	0.11	57.01	6.15E-06
Sulfur_Metabolism	0.02	0.53	0.07	0.019	0.25	**0**	0.04	0.02	**0**	75.59	5.00E-09
Aminoacyl_tRNA_Biosynthesis	0.13	0.16	0.12	**0**	0.66	**0**	0.013	**0.001**	0.02	79.60	1.01E-09
Glycosphingolipid_Biosynthesis_Ganglio_Series	0.28	0.011	0.21	**0**	0.24	**0**	0.03	0.03	**0.006**	78.07	1.85E-09
Limonene_and_Pinene_Degradation	**0.005**	0.62	0.03	**0**	0.45	**0**	0.07	0.011	0.02	78.41	1.62E-09
Pantothenate_and_Coa_Biosynthesis	0.26	0.94	0.08	**0**	0.02	**0.005**	0.26	0.02	**0.001**	68.28	8.78E-08
Citrate_Cycle_TCA_Cycle	0.03	0.17	0.05	0.014	0.72	**0**	0.016	**0**	**0**	88.59	2.58E-11
Valine_Leucine_and_Isoleucine_Biosynthesis	0.26	0.97	0.12	0.02	0.27	**0**	0.089	**0**	**0**	76.55	3.41E-09

*C p_value: Combined P value

T: test statistic following a chi-squared distribution

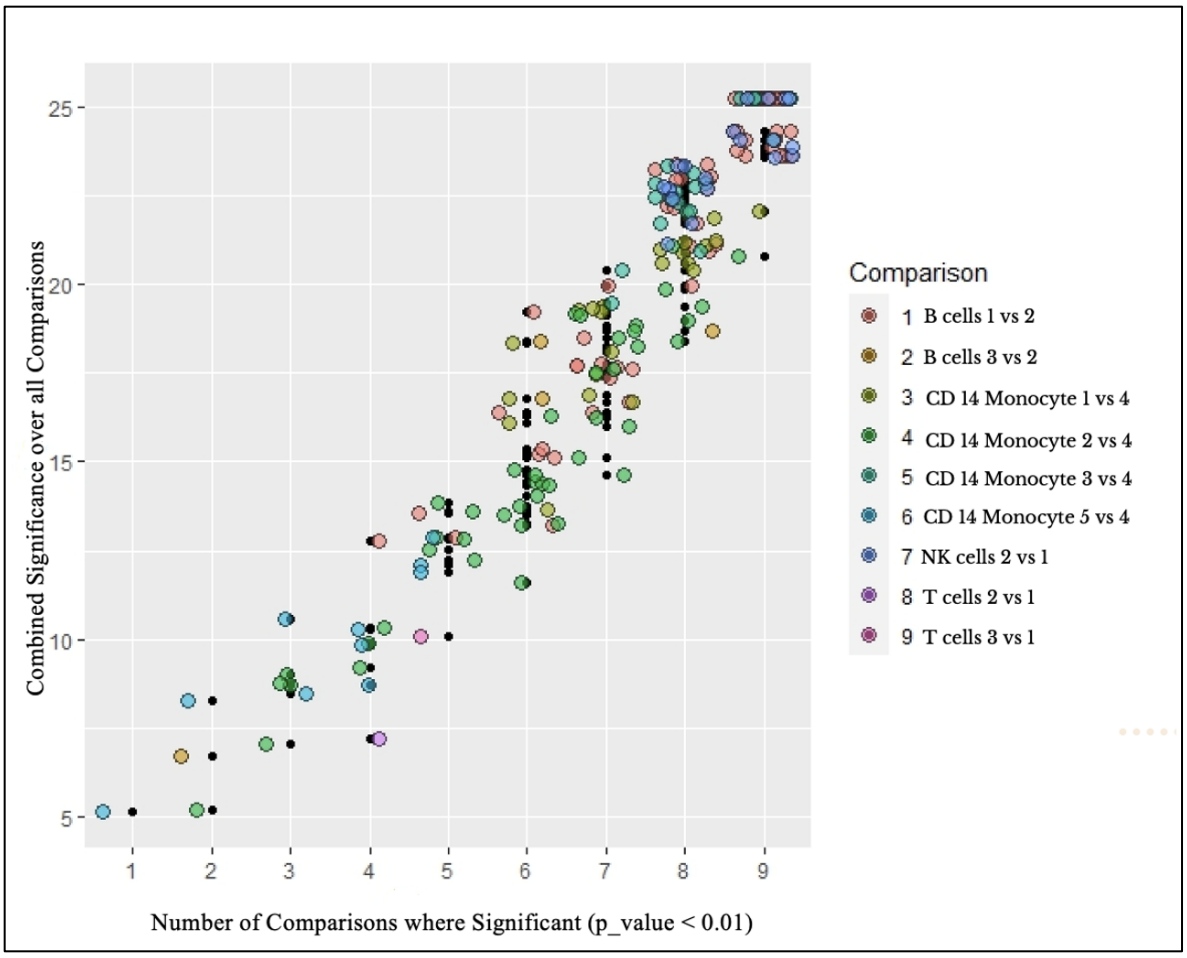

Figure 3. 1 Jitter Plot of Step 3 Analysis; combined significance over all comparisons vs. total number of comparisons with p_value < 0.01. *Black dots represent 186 gene set of KEGG. X[g] = Number of Comparisons where Significant (p_value <0.01). Y[g]= - log combined p_value. Color indicates the comparison with the smallest p_value.*

Table 3. 3 Differentially Expressed KEGG Pathways in Covid-19-Infected Samples Considering Comparisons with Minimum Combined p_values. *X[g] = Number of Comparisons where Significant (p_value < 0.01), and Y[g]= Combined Significance over all Comparisons.*

Gene set name	*X[G]*	*Y[G]*	*Group*
Taurine_and_Hypotaurine_Metabolism	1	5.13	CD14monocyt5_4
Non_Homologous_End_Joining	2	6.74	B cell3_2
			T cell3_1
Glycosphingolipid_Biosynthesis_Globo_Series	2	5.21	CD14monocyt2_4
			CD14monocyt3_4
Sulfur_Metabolism	2	8.3009	CD14monocyt5_4
			Tcell3_1
Aminoacyl_tRNA_Biosynthesis	3	8.99	Tcell2_1
			CD14monocyt2_4
			CD14monocyt5_4
Glycosphingolipid_Biosynthesis_Ganglio_Series	3	8.73	Tcell3_1
			CD14monocyt2_4
			CD14monocyt5_4
Limonene_and_Pinene_Degradation	3	8.78	B cell1_2
			CD14monocyt2_4
			CD14monocyt5_4
Pantothenate_and_CoA_Biosynthesis	3	7.05	Tcell3_1
			CD14monocyt2_4

			CD14monocyt5_4
Citrate_Cycle_TCA_Cycle	3	10.58	Tcell2_1
			Tcell3_1
			CD14monocyt5_4
Valine_Leucine_and_Isoleucine_Biosynthesis	3	8.46	Tcell2_1
			Tcell3_1
			CD14monocyt5_4

According to Table 3. 3, several gene sets related to amino acid and lipid biosynthesis and metabolism are differentially expressed within specific pairwise comparisons.

In CD14 monocytes and T cells, epigenetic signatures showed differential expression of KEGG pathways associated with sulfur metabolism, aminoacyl tRna_biosynthesis, glycosphingolipid biosynthesis; pantothenate and coAbiosynthesis, and citrate/TCA_cycle, indicating widespread alteration in amino acid and lipid biosynthesis and metabolism. In B and T cells, KEGG clusters with epigenetic signatures included non-homologous end joining, – important for repairing DNA double-stranded break (DSBs).

Chapter 4: Discussion, Limitations, and Conclusion

Our results suggest that Maternal COVID-19 may alter several amino acid biosynthesis and metabolism pathways in umbilical cord blood cells, potentially predisposing the fetus to metabolic syndrome conditions such as cardiovascular disease.

Previous studies demonstrated amino acid biosynthesis alteration during infection with COVID-19. Dysregulation of one-carbon metabolism is implicated in various biological processes and is linked to several diseases, including cardiovascular disease. Emerging evidence suggests that one-carbon metabolism plays a significant role in COVID-19. Numerous amino acids and their derivatives are integral to one-carbon metabolism. Following infection with the SARS-CoV-2 isolate (in African Green Monkey Vero E6 cells), intracellular levels of methionine (Met), cystathionine, pyridoxine, betaine, serine, glycine (Gly), 5-oxoproline (pyroglutamate), and cysteine-glutathione disulfide were reduced. In contrast, levels of reduced glutathione were elevated. However, intracellular levels of S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), cysteine (Cys), and oxidized glutathione (GSSG) remained unaffected in the SARS-CoV-2-infected cells. So, the SARS-CoV-2 virus hijacks many aspects of host sulfur amino acid metabolism, particularly glutathione metabolism involved in antioxidant defenses (Perła-Kaján & Jakubowski, 2022; Zhang et al., 2021). Metabolomic analysis of urine samples from COVID-19 and non-COVID-19 patients unveiled five pathways significantly up-regulated in COVID-19 patients. Among these pathways were taurine and hypotaurine metabolism as well as sulfur metabolism (Liu et al., 2021). Another comprehensive study aimed to delineate the independent and overlapping metabolic characteristics of samples from acutely ill patients, comprising 831 individuals who tested positive (n = 543) or negative (n = 288) for COVID-19. Remarkable alterations in amino acid metabolism emerged as highly pertinent

indicators of disease severity, progression, and prognosis concerning biological and clinical variables in these patients. Spearman correlation analyses revealed clinical and metabolic associations with coagulation parameters, notably highlighting sulfur metabolism, particularly cystine (D'Alessandro et al., 2021). Similarly, our findings suggest that several amino acid biosynthesis and metabolism genes are altered due to maternal COVID-19 infection.

The altered amino acid and lipid biosynthesis and metabolism pathways in umbilical cord blood may increase the risk of metabolic disease, including cardiovascular disease, in fetuses born from mothers infected with COVID-19. Atherosclerosis (AS) represents a progressive multifactorial immunomodulatory metabolic disease and serves as a precursor to various cardiovascular conditions, encompassing myocardial infarction, stroke, and angina pectoris (Bhattacharya et al., 2022). Endothelial cells, leukocytes, and smooth muscle cells within the intima play pivotal roles in the progression of this condition. During the initial stages of atherogenesis, an early cellular response involves the targeted recruitment of circulating monocytes, and a lesser involvement of T lymphocytes. Within the intima, monocytes undergo differentiation into macrophages and uptake atherogenic lipoproteins. The formation of lipid-laden macrophages, characterized by a significant accumulation of cholesteryl esters (foam cells), is a distinguishing feature of early and advanced atherosclerotic lesions. Sustained exposure to atherogenic lipoproteins leads to continuous ingestion by macrophages until their demise. The death of macrophages through apoptosis and necrosis creates a soft and destabilizing lipid-rich core within the plaque. Plaque rupture represents the primary cause of coronary thrombosis, as about 76% of fatal coronary thrombi occur due to the rupture of plaques. The characteristics of ruptured plaques include a substantial lipid-rich core, a thin fibrous cap with sparse smooth muscle cells and numerous macrophages, angiogenesis, inflammation in the adventitia, and outward remodeling (Falk, 2006;

Glass & Witztum, 2001; Hansson, 2005; Houtkamp et al., 2001; Libby, 2002; Spagnoli et al., 2007). In one study, uncovering potential metabolic signatures indicative of atherosclerosis (AS) in patients, a comprehensive analysis was conducted on 200 serum samples obtained from individuals diagnosed with AS and those with normal serum profiles. A distinct group of metabolites related to bile acids, amino acids, steroid hormones, and purine metabolism were identified. Furthermore, the targeted metabolomics approach corroborated these findings, revealing significant upregulation of six metabolites, including isoleucine, and significant downregulation of the concentrations of leucine and valine in the AS-risk sera. It was observed that pathways associated with amino acid biosynthesis played a crucial role in distinguishing between normal and AS sera, further underlining the significance of altered amino acid metabolism in the context of AS (Sardar et al., 2023). In another study, an integrated genomic analysis of two public datasets (GSE28829 comprises 16 carotid artery segment samples from patients with atherosclerosis and 13 normal control samples, and GSE43292 comprises 32 atherosclerosis samples and 32 control samples) was conducted to explore potentially important genes, key modules, infiltrating immune cells, and pathways involved in the pathogenesis of atherosclerosis. Regarding KEGG pathways, the disease group showed significantly higher expression of genes associated with limonene and pinene degradation and the valine leucine and isoleucine biosynthetic pathways while exhibiting lower expression of the glycosphingolipid biosynthesis globo series genes (Ji et al., 2023). Atherosclerotic lesions within the coronary arteries lead to narrowing or complete blockage of the vessel lumen. This constriction results in inadequate blood supply to the heart muscle, leading to myocardial ischemia, hypoxia, or necrosis (Malakar et al., 2019). Past investigations have firmly established that disruptions in metabolic pathways, such as elevated blood lipid levels and diabetes, constitute prominent risk factors contributing to the development of coronary artery disease (CAD). The study analyzed the

canonical correlation between CAD and gene expressions associated with metabolic pathways. The findings revealed that specific KEGG pathways, such as the tricarboxylic acid (TCA) cycle andglycosphingolipid biosynthesis ganglio series genes“ exhibited the most significant correlations with CAD genes (Lu et al., 2018). The glycosphingolipids synthesized via this pathway have been shown to accumulate in the artery wall, a recognized hallmark of atherosclerosis (Hara & Taketomi et al., 1991). Metabolomics offers a means to uncover novel intermediate metabolites, aiding in comprehending the underlying mechanisms contributing to the risk of atherosclerotic cardiovascular disease (ASCVD). A survey conducted on Surveillance of Risk Factors of Non-Communicable Diseases in Iran selected a random sample of 1,102 individuals aged between 40 and 79 years with LDL levels below 190 mg/dl and without pre-existing coronary artery disease or myocardial infarction. Utilizing the 10-year ASCVD risk score, participants were categorized into four groups based on their ASCVD risk level: low, borderline, intermediate, and high. Fasting plasma samples from these individuals were analyzed for thirty acylcarnitines and twenty amino acids. The identified differential metabolites between the low ASCVD risk group and the borderline/intermediate/high ASCVD risk groups were predominantly associated with various biological processes, including aminoacyl tRNA biosynthesis and the biosynthesis of valine leucine and isoleucine. Notably, the valine, leucine, and isoleucine biosynthesis pathway exhibited the highest enrichment ratio, elucidating the differences in metabolic profiles among the ASCVD risk groups. Previous studies have highlighted the involvement of these mechanisms in cardiovascular event development. For example, aminoacyl tRNA biosynthesis has been implicated in regulating the function of regulatory proteins across different cellular processes. Their impact on coronary arteries, aorta, cardiomyocytes, fibroblasts, and their association with angiogenesis and cardiomyopathy underscores the potential of this pathway as therapeutic or diagnostic targets for ASCVDs. Additionally, disruptions in the

mechanisms involving valine, leucine, isoleucine, and their α-keto acids have been observed in animals experiencing cardiac events (Dehghanbanadaki et al., 2023). Our results also indicate differential expression of these pathways in specific immune cells of the umbilical cord blood of COVID-19-infected-mothers, suggesting a potential future risk of atherosclerosis for the fetus.

Another microarray dataset, GSE20129, comprising 118 samples from the peripheral blood of female patients (47 atherosclerotic and 71 non-atherosclerotic patients), was utilized to identify differentially expressed genes (DEGs) associated with atherosclerosis. Employing the Recursive Feature Elimination (RFE) algorithm, 11 biomarkers, including CSAD, were identified. Cysteine sulfinic acid decarboxylase (CSAD) is an enzyme that catalyzes the conversion of cysteine sulfinic acid to hypotaurine, potentially limiting taurine production. Taurine, 2-aminoethanesulfonic acid, has been demonstrated to possess protective effects against cardiovascular diseases and can effectively impede the progression of atherosclerotic diseases (Wójcik et al., 2010). Therefore, it is hypothesized that CSAD may play a role in the advancement of atherosclerosis by modulating the generation of taurine (Chang et al., 2004; Liu et al., 2016). Reduction of cellular senescence, protection against telomerase deficiency, inhibition of mitochondrial dysfunction, reduction of DNA damage, mitigation of inflammation, and modulation of stress are among taurine's advantageous biological effects (Ito et al., 2014; Preising et al., 2019; Ripps & Shen, 2012; Singh et al., 2023; Warskulat et al., 2007). Taurine, essential for blood cell composition, is critical in maintaining hematologic function. Taurine plays a role in the synthesis, growth, differentiation, maturation, stabilization, optimal functioning, and programmed cell death of blood cells. In neutrophils and monocytes, taurine exhibits antioxidative properties by interacting with hypochlorous acid, forming taurine chloramine (TauCL). Taurine chloramine, being a milder oxidant, enhances phagocytosis and retards cellular apoptosis. TauCl is released from activated

phagocytes upon their apoptosis and inhibit the production of inflammatory mediators. These include superoxide anion, nitric oxide, tumor necrosis factor-α, interleukins, and prostaglandins in inflammatory cells within inflamed tissues. Thus, a key function of TauCl is initiating inflammation resolution. Moreover, it acts as a protective mechanism, safeguarding macrophages and surrounding tissues from damage caused by cytotoxic reactive oxygen metabolites that are excessively produced during inflammation (Kim & Cha, 2014). Increasing evidence indicates a potential causal relationship between immunosenescence and age-related chronic diseases, including susceptibility to atherosclerotic cardiovascular disease (ASCVD) (Del Pinto & Ferri, 2018; Laderoute, 2015; Laderoute, 2020; Müller et al., 2019; Oishi & Manabe, 2016). Immunosenescence, which refers to the aging-related decline in immune function, impacts innate and adaptive immunity. Monocytes and macrophages (M/M) play central roles in orchestrating immune responses, making them key targets and mediators of immunosenescence. This condition, influenced by age, stress, and persistent infections, results in immunosuppressed semi-activated macrophages that are unable to differentiate appropriately. Concurrently, these cells release pro-inflammatory cytokines in an unregulated manner. Consequently, these dysfunctional cells accumulate in tissues, originating from their earlier role as monocytes, and contribute disease development. A recent meta-analysis of randomized controlled trials (RCTs) indicates that taurine supplementation may yield favorable effects on specific cardiometabolic indicators in individuals with underlying comorbidities such as diabetes. The analysis reveals that taurine supplementation led to reductions in systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), and triglycerides (TG) levels (Guan & Miao, 2020). Exposure to taurine during the perinatal period can have more enduring effects on adult function and susceptibility to diseases such as cardiovascular disease, compared to exposure during adulthood (Roysommuti & Wyss, 2014). Intrauterine growth restriction resulting in low birth weight is widely recognized as a leading

cause of adverse neonatal outcomes associated with taurine deficiency (Norberg et al., 1998), predisposes newborns to conditions such as diabetes mellitus, obesity, and hypertension in adulthood (Roysommuti & Wyss, 2014). Moreover, numerous researchers have found that supplementing adult offspring deprived of taurine prenatally and/or early postnatally can prevent several disorders. Taurine or taurine-rich diet may prevent or alleviate hypertension in both animal and human models. In adult spontaneously hypertensive rats, taurine supplementation, for example, has been demonstrated to reduce hypertension. Furthermore, taurine therapy appears to shield against hypertension brought on by a diet high in sugar. According to epidemiological research, people who consume much taurine in their diet also typically have lower rates of hypertension and other cardiovascular illnesses. (Roysommuti & Wyss, 2014; Yamori et al., 2010). Targeted metabolomics analyses conducted on sera from forty-nine subjects, comprising 33 COVID-19–positive individuals and 16 COVID-19–negative subjects, revealed significant associations between COVID-19 status and interleukin-6 (IL-6) levels with amino acid metabolism. Notably, sulfur-containing amino acids such as cysteine and taurine tended to decrease, particularly in the moderate-high IL-6 group. Conversely, oxidized forms of sulfur-containing amino acids like methionine sulfoxide, cystine, and arginine, exhibited increases. Although no significant alterations were observed in methionine levels, elevations in acetyl-methionine and hydroxyproline were noted, especially in COVID-19 patients with the highest IL-6 levels (Thomas et al., 2020). Recent progress in understanding monocyte diversity has underscored the importance of different monocyte subsets in hypercholesterolemic mice. These subsets exhibit specific recruitment to early lesions, adipose tissue, or infarcted myocardium (Swirski et al., 2007). However, the exact role of each monocyte subset in atherogenesis remains to be fully elucidated. Similarly, our results demonstrated differential expression of KEGG gene sets involved in the pathophysiology of atherosclerosis in specific subsets of CD 14 Monocytes.

Similarly, our findings revealed that taurine and hypotaurine metabolism pathway is only differentially expressed in a specific cluster of CD 14 monocytes, showing epigenetic regulation. This suggests that through epigenetic modulation, metabolism of taurine and hypotaurine in CD14 monocytes can be disturbed, which may affect its proper differentiation and increase the risk of immunosenescence disease like ASCVD in fetuses born from COVID-19-infected-mothers.

According to a study conducted by Verschuren et al., the non-homologous end-joining (NHEJ) pathway was found to be associated with the occurrence of MI in both the GENDER study (P = 0.0083) and the PROSPER study (P = 0.014) (Verschuren et al., 2013) (The GENetic DEterminants of Restenosis (GENDER) study, 3,104 consecutive unrelated symptomatic patients who were successfully treated with percutaneous coronary intervention (PCI) for angina were included. The study conducted a follow-up period of 9 months to analyze the combination of prevalent and incident myocardial infarction (MI) or stroke. PROSPER is a prospective multicenter randomized placebo-controlled trial designed to evaluate whether treatment with pravastatin reduces the risk of major vascular events in elderly individuals aged 70–82 years in Scotland (Glasgow), Ireland (Cork), and The Netherlands (Leiden) between December 1997 and May 1999). Atherosclerotic plaques, triggers of myocardial infarction and stroke, are characterized by the accumulation of vascular smooth muscle cells (VSMCs) and inflammatory cells, along with lipid and extracellular matrix proteins (Dutta et al., 2012). Despite the chronic inflammatory nature of plaque development, growing evidence indicates that DNA damage to cells within the lesion plays a significant role in both atherogenesis and the behavior of established lesions (Mahmoudi et al., 2006). Our results suggest that in addition to differential expression of other pathways involved in atherosclerosis, non-homologous end joining (NHEJ) is differentially expressed in specific subsets of T and B cells, which means maternal SARS-CoV-2 infection may

lead to DNA damage in these cell types which can promote atherogenesis**.** A possible explanation could be oxidative DNA damage. Reactive oxygen species (ROS) continuously generated within cells during pathological processes, such as inflammation (Williams & Jeffrey, 2000). Infections with RNA viruses, such as SARS-CoV-2, have been associated with elevated production of free radicals and increased consumption of antioxidants, leading to dysregulation of Oxidative Stress (OxS) in both the mother and placenta (Moreno-Fernandez et al., 2022; Rolfo et al., 2022; Tepebaşı et al., 2022)

Maternal Covid-19 may predispose fetuses to other cardiovascular disease such as dilated cardiomyopathy and atrial fibrillation. Dilated cardiomyopathy (DCM) is a primary cardiomyopathy with an unknown cause, commonly affecting both children and older adults. Despite its prevalence, the lack of noticeable symptoms and identifiable biomarkers presents challenges in the early detection and management of DCM. two datasets were utilized to identify potential diagnostic markers for DCM: GSE120895, consisting of 47 DCM patients and 8 controls, and GSE9800, comprising 12 DCM patients and 6 controls. To investigate the functional differences between DCM and control samples, Gene Set Variation Analysis (GSVA) was employed to assess the relative expression variances of pathways in the two groups. The GSVA analysis revealed significant enrichment of various differentially expressed pathways. Notably, in comparison to the control groups, there was a marked decrease in the expression of pathways associated with valine leucine and isoleucine biosynthesis pathway in the DCM group (Zhou et al., 2021). Atrial fibrillation (AF) represents one of the most prevalent types of arrhythmias, often associated with significant clinical complications. To gain deeper insights into the metabolomic profile of AF and identify potential biomarkers for AF diagnosis or prediction, a study was conducted utilizing atrial appendage samples from sixty individuals (30 with AF and 30 without

AF), and plasma samples from 49 AF patients and 116 non-AF patients. Among the 24 metabolites analyzed in the atrial appendage samples, eight metabolites, including L-valine and taurine, exhibited a substantial difference, mainly showing increased levels in the AF group compared to the non-AF group. In the analysis of plasma samples, 24 metabolites were examined. Among these, nine metabolites, including L-valine, demonstrated a notable increase in levels in the AF group compared to the non-AF group. Conversely, seven metabolites, including L-leucine, exhibited significantly decreased concentrations in the AF group compared to those in the non-AF group. L-valine consistently displayed elevated levels across all samples in both the atrial appendage and the plasma samples. The KEGG analysis of the differential metabolites in the atrial appendage samples revealed seven significantly altered pathways, which included aminoacyl tRNA biosynthesis and taurine and hypotaurine metabolism and the analysis of plasma samples identified six significantly altered pathways, with valine leucine and isoleucine biosynthesis being one of them (Lai et al., 2018).

4.1 Other Possible Fetal Complications

Extracellular vesicles (Evs) are increasingly recognized as vital components in the communication between the embryo and maternal tissues under physiological and pathological conditions. These Evs act as carriers, facilitating the transfer of nucleic acid, proteins, and lipids between cells, thereby enabling intercellular communication without direct cell-to-cell contact. Glycosphingolipids (GSLs) in Evs have emerged as promising mediators of cross-boundary cell-to-cell communication (Lo et al., 2023; Tannetta et al., 2014). Whether differential expression of glycosphingolipid biosynthesis in umbilical cord blood mononuclear cells of COVID-1- infected-

mothers can disturb intercellular communication between the mother and the fetus through Evs needs further investigation.

Effective DNA repair is essential for the survival of an organism, given that DNA is constantly exposed to diverse exogenous factors such as chemicals, radiation, and internally generated triggers like reactive oxygen species (ROS) and DNA replication errors, all of which can cause DNA damage. Human cells have multiple intrinsic DNA repair mechanisms to safeguard against the detrimental effects of DNA damage (Ozturk & Demir, 2011). To repair double-strand breaks (DSBs) that are considered to carry the highest risk of triggering deleterious events such as chromosomal translocations, cancer, and cell death, repair mechanisms such as non-homologous end-joining (NHEJ) are involved (Waters et al., 2009). NHEJ operates very quickly, with half-times typically ranging from 10 to 30 minutes and is essentially error prone. It functions by directly joining the ends of DNA strands without the ability to restore the original sequence in the vicinity of the double-strand break (DSB). It lacks built-in mechanisms to ensure the restoration of the original DNA molecule. In principle, it can join any DNA ends regardless of their molecular origin, which can manifest chromosomal translocations (Dueva & Iliakis, 2013). Several studies suggest that DSBs and their repair by NHEJ play a role in the formation of critical chromosomal translocations observed in both childhood and adult acute myeloid and lymphoid leukemias (Rassool, 2003) (Elliott & Jasin, 2002) (Preston et al., 1994) (Pierce et al., 1996; Wiemels et al., 2000). Whether and how differential expression of NHEJ in B and T cells of umbilical cord blood cells of COVID-19-infected-mothers may increase the likelihood of developing lymphoid leukemias in fetuses is of significant concern, and further investigations are warranted.

Gene expression analysis has evolved significantly and remains essential for understanding biological systems. Measuring RNA and protein levels offers unique insights into cell functions. Advanced technologies like high-throughput next-generation sequencing (NGS) have made gene expression analysis more efficient and popular in modern research. Different techniques have distinct advantages and limitations depending on research goals, available resources, and sensitivity requirements. Bulk NGS provides a comprehensive view of cell clusters but may overlook cellular diversity. Single-cell RNA sequencing (scRNAseq) addresses this limitation by examining individual cells, allowing for the study of cellular diversity and developmental relationships in various disease conditions. We used the available gene expression data set (Gene Expression Omnibus database (GSE 165193). Matute et al. employed droplet-based single cell RNA sequencing (scRNAsq) in this study, a cutting-edge method in high-throughput next-generation sequencing.

In this study we focused on gene set level analysis instead of the individual gene analysis conducted by authors. Gene set level analysis offers several advantages over gene level analysis in interpreting high-dimensional gene expression data. By concentrating on predefined sets of functionally related genes rather than individual genes, gene set analysis allows for a more biologically meaningful interpretation of complex datasets. This approach provides a broader perspective by considering the coordinated activity of genes within biological pathways or functional modules, which can uncover subtle but coordinated changes in gene expression that may be missed by examining individual genes in isolation. Additionally, gene set analysis enhances statistical power by aggregating information across multiple genes, thereby reducing the impact of noise and variability inherent in high-dimensional data. Gene set level analysis plays a crucial role in uncovering potential therapeutic targets and preventive methods for various

diseases. Gene set analysis can identify key pathways that are dysregulated in disease states by examining the collective behavior of functionally related genes within biological pathways or processes. Dysregulated pathways represent potential targets for therapeutic intervention, as modulating their activity could restore normal cellular function and mitigate disease progression. Additionally, gene set analysis can reveal pathways associated with disease risk, such as the KEGG pathways we suggest in this study, providing insights into potential preventative strategies. By understanding the underlying molecular mechanisms involved in disease development, researchers can develop targeted therapies to restore normal pathway activity or prevent the onset of disease by modulating relevant biological processes. Furthermore, gene set analysis enables the identification of biomarkers associated with disease risk or treatment response, which can inform personalized treatment strategies and facilitate the development of precision medicine approaches. Overall, gene set level analysis serves as a valuable tool in drug discovery and development, offering insights into disease mechanisms and guiding the development of novel therapies and preventive measures.

One particularly effective method for gene set analysis is the Linear Combination Test (LCT). This method, with its ability to efficiently capture the intrinsic geometric structure of the data and identify gene sets that exhibit coordinated expression patterns, is a reliable and robust tool. It is particularly suited for analyzing high-dimensional gene expression data, providing a solid framework for identifying biologically relevant pathways or processes associated with the observed phenotypes. In summary, gene set level analysis, particularly when coupled with robust methods like LCT offers a comprehensive and interpretable approach for uncovering meaningful insights from complex gene expression datasets.

Matute et al., 2022 have identified several limitations that warrant discussion. Notably, the small sample size restricts the extent to which conclusions can be generalized. Additionally, it's crucial to recognize that all cases included in the study involved mothers with mild SARS-CoV-2 infection; more severe cases could potentially yield different or more pronounced fetal immune genomic signatures, particularly considering evidence suggesting an increased likelihood of intensive care unit admission with more severe maternal infection during pregnancy and increased risk of NICU admission for neonates. Moreover, the interval between maternal infection and delivery, as well as cord blood collection, may influence the observed immune phenotype in cord blood. Given the variation in timing between infection and collection within our cohort (ranging from 7 to 66 days), more consistent timing could potentially yield more significant findings. Lastly, it's important to acknowledge that all mothers affected by SARS-CoV-2 had comorbidities such as well-controlled thyroid dysfunction, obesity, or gestational diabetes. While efforts were made to include mothers with similar comorbidities in the control population (apart from gestational diabetes), and all comorbidities were managed medically, it's plausible that our results may be influenced by maternal comorbidities. However, it's worth noting that thyroid disease, obesity, or gestational diabetes in the mother have not been reported to elicit the transcriptional response patterns observed in cases compared to controls.

While our study addresses certain limitations of the original published paper, it is imperative to approach the results with caution. Although gene expression data offer valuable insights into transcriptional activity, they may not comprehensively capture epigenetic modifications that contribute to the development of metabolic syndrome in neonates. Supplementing our analysis with additional methodologies, such as DNA methylation profiling using Bisulfite Sequencing or investigating histone modifications via Chromatin Immunoprecipitation (ChIP), could yield a

more thorough understanding of epigenetic programming in this context. Furthermore, during pregnancy, a multitude of factors can influence epigenetic modifications, thereby impacting fetal development and long-term health outcomes. These factors include maternal diet, psychological, environmental, or socio-economic stress, lifestyle, health conditions, advanced age, and variations in the gut microbiome. It is not feasible to match cases and controls neither in this study nor other studies regarding all the factors contributing to epigenetic modulation thus we need to interpret the results with careful attention. In addition, the growth and development of the fetus rely heavily on the mother. The placenta serves as the crucial link between the mother and the fetus, facilitating fetal development and providing early life immunity through the transfer of growth factors, nutrients, oxygen, and pathogen-specific antibodies, and maternal microchimeric immune cells (MMc) (Stelzer et al., 2021). In this study authors did not employ a strategy to exclude maternal side of the umbilical cord blood mononuclear cells which may affect the results.

In this study, we used the KEGG collection, a comprehensive and widely used collection of biological pathways, to assess altered canonical biological pathways due to maternal COVID-19 in CBMCs. The KEGG database, is however, not the only database providing biological pathways, and there is no consensus on the best database. Other examples are Reactome, BioCarta, WikiPathways, Panther (Protein ANalysis THrough Evolutionary Relationships) Pathways, and MPDB (Small Molecule Pathway Database). By exploring and integrating data from these additional pathway collections into our research, we can broaden the scope of our analysis and gain deeper insights into the biological processes relevant to our research interest. Additionally, considering multiple pathway databases allows for cross-validation of findings and enhances the robustness of the research conclusions. It is important to consider that probably none of these

databases perfectly represent the actual biological mechanism, simply because our current knowledge has yet to be far evolved.

Utilizing 'omics technologies across diverse platforms (genome, transcriptome, proteome, metabolome, microbiome, lipidome), along with extensive high-dimensional population databases and distinct cohorts, can offer comprehensive insights into the precise impact of maternal COVID-19 on the long-term health outcomes of neonates. Conducting studies on metabolomics and gene expression simultaneously can help to better understand health outcomes by providing complementary information about biological processes and pathways. By examining the expression patterns of sets of genes, we can understand how these pathways are regulated and dysregulated in terms of health and disease. This information can help identify key molecular mechanisms underlying health conditions and predict disease progression or treatment response. Metabolomics focuses on comprehensively analysing small molecules (metabolites) in biological samples. Metabolites are the end products of cellular processes and reflect the biochemical activity within cells and tissues. By profiling metabolites, metabolomics provides a snapshot of the metabolic state of an organism, offering insights into its physiological status, metabolic pathways, and interactions with environmental factors. Changes in metabolite levels can serve as biomarkers for disease diagnosis, prognosis, and monitoring treatment response. Integrating gene set level data and metabolomics allows a more comprehensive understanding of health outcomes. By correlating changes in gene expression with alterations in metabolite profiles, we can unravel intricate molecular networks and pathways associated with health and disease. This integrated approach enables the identification of biomarkers with improved diagnostic and prognostic accuracy and facilitates the development of targeted therapeutic interventions tailored to individual patients' metabolic and genetic profiles. Prospective cohorts allow for tracking

offspring health outcomes over time involving large sample sizes and extended follow-up periods, providing sufficient statistical power to detect meaningful associations between maternal COVID-19 infection and offspring cardiovascular outcomes later in life. This enhances the reliability and generalizability of study findings. Also, prospective cohorts enable the comprehensive assessment of potential confounding and cardiovascular disease risk factors. This includes detailed information on lifestyle factors, medical history, socioeconomic status, and genetic predispositions, which can help identify and adjust for potential confounders.

Evaluating cardiovascular complications arising from maternal COVID-19 can be further validated through both in vitro and in vivo investigations. Utilizing animal models like transgenic mice or non-human primates serves to corroborate the in vivo significance of identified pathways. This involves inducing maternal COVID-19 infection in pregnant animals and assessing its impact on offspring cardiovascular health through physiological, histological, and molecular analyses, such as assessing immune infiltration in carotid segments. Additionally, conducting pharmacological interventions targeting specific pathway components identified in our study provides further validation. Administering these drugs to animal models of maternal COVID-19 allows for evaluating their effects on offspring cardiovascular outcomes. Furthermore, genetic manipulations such as knockout or knockdown experiments targeting genes within identified pathways in animal models enable assessment of the consequences of pathway perturbations on offspring cardiovascular phenotypes and disease susceptibility.

In conclusion, comprehending the intricate interplay between maternal factors and epigenetic processes throughout pregnancy is pivotal for identifying modifiable risk factors and devising interventions to promote optimal fetal development and lifelong health. The integration of multi-

omics approaches, and longitudinal studies can aid in unraveling the mechanisms underlying maternal programming of epigenetic changes and their implications for the health and susceptibility of offspring to diseases.

Bibliography

Abu-Raya, B., Kollmann, T. R., Marchant, A., & MacGillivray, D. M. (2016). The immune system of HIV-exposed uninfected infants. *Frontiers in immunology*, *7*, 383.

Al-Haddad, B. J., Jacobsson, B., Chabra, S., Modzelewska, D., Olson, E. M., Bernier, R., Enquobahrie, D. A., Hagberg, H., Östling, S., & Rajagopal, L. (2019). Long-term risk of neuropsychiatric disease after exposure to infection in utero. *JAMA psychiatry*, *76*(6), 594-602.

Al-Haddad, B. J., Oler, E., Armistead, B., Elsayed, N. A., Weinberger, D. R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., & Wang, C. (2019). The fetal origins of mental illness. *American journal of obstetrics and gynecology*, *221*(6), 549-562.

Alfaraj, S. H., Al-Tawfiq, J. A., & Memish, Z. A. (2019). Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection during pregnancy: Report of two cases & review of the literature.

Allen, D. W., Kim, K. W., Rawlinson, W. D., & Craig, M. E. (2018). Maternal virus infections in pregnancy and type 1 diabetes in their offspring: Systematic review and meta-analysis of observational studies. *Rev Med Virol*, *28*(3), e1974. https://doi.org/10.1002/rmv.1974

Allotey, J., Stallings, E., Bonet, M., Yap, M., Chatterjee, S., Kew, T., Debenham, L., Llavall, A. C., Dixit, A., Zhou, D., Balaji, R., Lee, S. I., Qiu, X., Yuan, M., Coomar, D., Sheikh, J., Lawson, H., Ansari, K., van Wely, M., . . . Thangaratinam, S. (2020). Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. *Bmj*, *370*, m3320. https://doi.org/10.1136/bmj.m3320

Anderson, D., Neri, J. I., Souza, C. R., Valverde, J. G., De Araújo, J. M., Nascimento, M. D., Branco, R. C., Arrais, N. M., Lassmann, T., & Blackwell, J. M. (2021). Zika virus changes methylation of genes involved in immune response and neural development in Brazilian babies born with congenital microcephaly. *The Journal of infectious diseases*, *223*(3), 435-440.

Apostol, A. C., Jensen, K. D., & Beaudin, A. E. (2020). Training the fetal immune system through maternal inflammation—a layered hygiene hypothesis. *Frontiers in immunology*, *11*, 123.

Arora, I., & Tollefsbol, T. O. (2021). Computational methods and next-generation sequencing approaches to analyze epigenetics data: Profiling of methods and applications. *Methods*, *187*, 92-103. https://doi.org/10.1016/j.ymeth.2020.09.008

Ather, A., Patel, B., Ruparel, N. B., Diogenes, A., & Hargreaves, K. M. (2020). Coronavirus disease 19 (COVID-19): implications for clinical dental care. *Journal of endodontics*, *46*(5), 584-595.

Atladóttir, H., Henriksen, T. B., Schendel, D. E., & Parner, E. T. (2012). Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics*, *130*(6), e1447-1454. https://doi.org/10.1542/peds.2012-1107

Barchetta, I., Arvastsson, J., Sarmiento, L., & Cilio, C. M. (2021). Epigenetic changes induced by maternal factors during fetal life: Implication for Type 1 Diabetes. *Genes*, *12*(6), 887. https://www.mdpi.com/2073-4425/12/6/887

Barcelos, I. D. E. S., de Araújo Penna, I. A., de Góes Soligo, A., Costa, Z. B., & Martins, W. P. (2021). Vertical transmission of SARS-CoV-2: a systematic review. *Revista Brasileira de Ginecologia e Obstetrícia/RBGO Gynecology and Obstetrics*, *43*(03), 207-215.

Barker, D. J. (1998). In utero programming of chronic disease. *Clin Sci (Lond)*, *95*(2), 115-128.

Barrès, R., & Zierath, J. R. (2016). The role of diet and exercise in the transgenerational epigenetic landscape of T2DM. *Nat Rev Endocrinol*, *12*(8), 441-451. https://doi.org/10.1038/nrendo.2016.87

Barua, S., & Junaid, M. A. (2015). Lifestyle, pregnancy and epigenetic effects. *Epigenomics*, *7*(1), 85-102. https://doi.org/10.2217/epi.14.71

Basil, P., Li, Q., Dempster, E., Mill, J., Sham, P., Wong, C., & McAlonan, G. (2014). Prenatal maternal immune activation causes epigenetic differences in adolescent mouse brain. *Translational Psychiatry*, *4*(9), e434-e434.

Bavaresco, T., Menolli, R. A., Frizon, B. J. Z., Viera, C. S., Conterno, J. R., Guimarães, A. T. B., & Grassiolli, S. (2019). Hypertension and maternal urinary tract infection and the metabolic conditions of preterm infants. *Revista Brasileira de Enfermagem*, *72*, 3-8.

Bergman, Y., & Cedar, H. (2013). DNA methylation dynamics in health and disease. *Nat Struct Mol Biol*, *20*(3), 274-281. https://doi.org/10.1038/nsmb.2518

Bermick, J., & Schaller, M. (2022). Epigenetic regulation of pediatric and neonatal immune responses. *Pediatric Research*, *91*(2), 297-327. https://doi.org/10.1038/s41390-021-01630-3

Berrar, D. P., Dubitzky, W., & Granzow, M. (2003). A practical approach to microarray data analysis. *Springer*.

Bhattacharya, P., Kanagasooriyan, R., & Subramanian, M. (2022). Tackling inflammation in atherosclerosis: Are we there yet and what lies beyond? *Current Opinion in Pharmacology*, *66*, 102283.

Bird, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature*, *321*(6067), 209-213.

Bohacek, J., & Mansuy, I. M. (2015). Molecular insights into transgenerational non-genetic inheritance of acquired behaviours. *Nat Rev Genet*, *16*(11), 641-652. https://doi.org/10.1038/nrg3964

Bošković, A., & Rando, O. J. (2018). Transgenerational Epigenetic Inheritance. *Annu Rev Genet*, *52*, 21-41. https://doi.org/10.1146/annurev-genet-120417-031404

Brown, A. S., Cohen, P., Harkavy-Friedman, J., Babulas, V., Malaspina, D., Gorman, J. M., & Susser, E. S. (2001). A.E. Bennett Research Award. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. *Biol Psychiatry*, *49*(6), 473-486. https://doi.org/10.1016/s0006-3223(01)01068-x

Brown, A. S., & Derkits, E. J. (2010). Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am J Psychiatry*, *167*(3), 261-280. https://doi.org/10.1176/appi.ajp.2009.09030361

Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y., & Greenleaf, W. J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nature Methods*, *10*(12), 1213-1218. https://doi.org/10.1038/nmeth.2688

Cai, J., Sun, W., Huang, J., Gamber, M., Wu, J., & He, G. (2020). Indirect virus transmission in cluster of COVID-19 cases, Wenzhou, China, 2020. *Emerging infectious diseases*, *26*(6), 1343.

Calay, E. S., & Hotamisligil, G. S. (2013). Turning off the inflammatory, but not the metabolic, flames. *Nat Med*, *19*(3), 265-267. https://doi.org/10.1038/nm.3114

Canetta, S. E., Bao, Y., Co, M. D., Ennis, F. A., Cruz, J., Terajima, M., Shen, L., Kellendonk, C., Schaefer, C. A., & Brown, A. S. (2014). Serological documentation of maternal influenza

exposure and bipolar disorder in adult offspring. *Am J Psychiatry*, *171*(5), 557-563. https://doi.org/10.1176/appi.ajp.2013.13070943

Carlos, W. G., Dela Cruz, C. S., Cao, B., Pasnick, S., & Jamil, S. (2020). Novel wuhan (2019-nCoV) coronavirus. *Am J Respir Crit Care Med*, P7-P8.

Cedergren, M. (2006). Effects of gestational weight gain and body mass index on obstetric outcome in Sweden. *Int J Gynaecol Obstet*, *93*(3), 269-274. https://doi.org/10.1016/j.ijgo.2006.03.002

Chambers, C., Krogstad, P., Bertrand, K., Contreras, D., Tobin, N. H., Bode, L., & Aldrovandi, G. (2020). Evaluation for SARS-CoV-2 in breast milk from 18 infected women. *JAMA*, *324*(13), 1347-1348. https://doi.org/10.1001/jama.2020.15580

Chambers, J. M., Cleveland, W. S., Kleiner, B. and Tukey, P.A. . (1983). *Graphical Methods for Data Analysis*.

Chambers, J. M. a. H., T. J. (1992). Statistical Models in S.

Chang, L., Xu, J., Yu, F., Zhao, J., Tang, X., & Tang, C. (2004). Taurine protected myocardial mitochondria injury induced by hyperhomocysteinemia in rats. *Amino acids*, *27*, 37-48.

Chen, H., Guo, J., Wang, C., Luo, F., Yu, X., Zhang, W., Li, J., Zhao, D., Xu, D., & Gong, Q. (2020). Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *The lancet*, *395*(10226), 809-815.

Chen, J., Liang, Y., Yi, P., Xu, L., Hawkins, H. K., Rossi, S. L., Soong, L., Cai, J., Menon, R., & Sun, J. (2017). Outcomes of congenital zika disease depend on timing of infection and maternal-fetal interferon action. *Cell Rep*, *21*(6), 1588-1599. https://doi.org/10.1016/j.celrep.2017.10.059

Chen, J., Wang, R., Hozumi, Y., Liu, G., Qiu, Y., Wei, X., & Wei, G. W. (2023). Emerging dominant SARS-CoV-2 variants. *J Chem Inf Model*, *63*(1), 335-342. https://doi.org/10.1021/acs.jcim.2c01352

Chen, P., Piaggi, P., Traurig, M., Bogardus, C., Knowler, W. C., Baier, L. J., & Hanson, R. L. (2017). Differential methylation of genes in individuals exposed to maternal diabetes in utero. *Diabetologia*, *60*, 645-655.

Choi, G. B., Yim, Y. S., Wong, H., Kim, S., Kim, H., Kim, S. V., Hoeffer, C. A., Littman, D. R., & Huh, J. R. (2016). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science*, *351*(6276), 933-939. https://doi.org/doi:10.1126/science.aad0314

Clausen, T. D., Mathiesen, E. R., Hansen, T., Pedersen, O., Jensen, D. M., Lauenborg, J., Schmidt, L., & Damm, P. (2009). Overweight and the metabolic syndrome in adult offspring of women with diet-treated gestational diabetes mellitus or type 1 diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *94*(7), 2464-2470.

Cocoros, N. M., Lash, T. L., Ozonoff, A., Nørgaard, M., DeMaria, A., Jr., Andreasen, V., & Sørensen, H. T. (2014). Prenatal influenza exposure and cardiovascular events in adulthood. *Influenza Other Respir Viruses*, *8*(1), 83-90. https://doi.org/10.1111/irv.12202

Cooney, C. A., Dave, A. A., & Wolff, G. L. (2002). Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J Nutr*, *132*(8 Suppl), 2393s-2400s. https://doi.org/10.1093/jn/132.8.2393S

Corujo, D., & Buschbeck, M. (2018). Post-translational modifications of H2A histone variants and their role in cancer. *Cancers*, *10*(3), 59.

Cui, X., & Churchill, G. A. (2003). Statistical tests for differential expression in cDNA microarray experiments. *Genome Biology*, *4*(4), 210. https://doi.org/10.1186/gb-2003-4-4-210

D'Alessandro, A., Thomas, T., Akpan, I. J., Reisz, J. A., Cendali, F. I., Gamboni, F., Nemkov, T., Thangaraju, K., Katneni, U., & Tanaka, K. (2021). Biological and clinical factors contributing to the metabolic heterogeneity of hospitalized patients with and without COVID-19. *Cells*, *10*(9), 2293.

Dahlquist, G. G., Ivarsson, S., Lindberg, B., & Forsgren, M. (1995). Maternal enteroviral infection during pregnancy as a risk factor for childhood IDDM: a population-based case-control study. *Diabetes*, *44*(4), 408-413. https://doi.org/10.2337/diab.44.4.408

Dashraath, P., Wong, J. L. J., Lim, M. X. K., Lim, L. M., Li, S., Biswas, A., Choolani, M., Mattar, C., & Su, L. L. (2020). Coronavirus disease 2019 (COVID-19) pandemic and pregnancy. *Am J Obstet Gynecol*, *222*(6), 521-531. https://doi.org/10.1016/j.ajog.2020.03.021

Davie, J. R. (2003). Inhibition of histone deacetylase activity by butyrate. *J Nutr*, *133*(7 Suppl), 2485s-2493s. https://doi.org/10.1093/jn/133.7.2485S

Dehghanbanadaki, H., Dodangeh, S., Parhizkar Roudsari, P., Hosseinkhani, S., Khashayar, P., Noorchenarboo, M., Rezaei, N., Dilmaghani-Marand, A., Yoosefi, M., & Arjmand, B. (2023). Metabolomics profile and 10-year atherosclerotic cardiovascular disease (ASCVD) risk score. *Frontiers in Cardiovascular Medicine*, *10*, 1161761.

Del Pinto, R., & Ferri, C. (2018). Inflammation-accelerated senescence and the cardiovascular system: mechanisms and perspectives. *Int J Mol Sci*, *19*(12). https://doi.org/10.3390/ijms19123701

del Rosario, M. C., Ossowski, V., Knowler, W. C., Bogardus, C., Baier, L. J., & Hanson, R. L. (2014). Potential epigenetic dysregulation of genes associated with MODY and type 2 diabetes in humans exposed to a diabetic intrauterine environment: an analysis of genome-wide DNA methylation. *Metabolism*, *63*(5), 654-660. https://doi.org/10.1016/j.metabol.2014.01.007

Deng, Y., Song, L., Nie, X., Shou, W., & Li, X. (2018). Prenatal inflammation exposure-programmed cardiovascular diseases and potential prevention. *Pharmacol Ther*, *190*, 159-172. https://doi.org/10.1016/j.pharmthera.2018.05.009

DesJarlais, R., & Tummino, P. J. (2016). Role of histone-modifying enzymes and their complexes in regulation of chromatin biology. *Biochemistry*, *55*(11), 1584-1599.

Dinu, I., Potter, J. D., Mueller, T., Liu, Q., Adewale, A. J., Jhangri, G. S., Einecke, G., Famulski, K. S., Halloran, P., & Yasui, Y. (2007). Improving gene set analysis of microarray data by SAM-GS. *BMC bioinformatics*, *8*, 242. https://doi.org/10.1186/1471-2105-8-242

Doenecke, D. (2014). Chromatin dynamics from S-phase to mitosis: contributions of histone modifications. *Cell Tissue Res*, *356*(3), 467-475. https://doi.org/10.1007/s00441-014-1873-1

Dong, A., Yoder, J. A., Zhang, X., Zhou, L., Bestor, T. H., & Cheng, X. (2001). Structure of human DNMT2, an enigmatic DNA methyltransferase homolog that displays denaturant-resistant binding to DNA. *Nucleic acids research*, *29*(2), 439-448.

Dong, L., Tian, J., He, S., Zhu, C., Wang, J., Liu, C., & Yang, J. (2020). Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *JAMA*, *323*(18), 1846-1848. https://doi.org/10.1001/jama.2020.4621

Draghici, S. (2002). Statistical intelligence: effective analysis of high-density microarray data. *Drug discovery today*, *7*(11), S55-S63.

Drosten, C., Meyer, B., Müller, M. A., Corman, V. M., Al-Masri, M., Hossain, R., Madani, H., Sieberg, A., Bosch, B. J., & Lattwein, E. (2014). Transmission of MERS-coronavirus in household contacts. *New England Journal of Medicine*, *371*(9), 828-835.

Dueva, R., & Iliakis, G. (2013). Alternative pathways of non-homologous end joining (NHEJ) in genomic instability and cancer. *Transl Cancer Res*, *2*(3), 163-177.

Dunn, G. A., & Bale, T. L. (2009). Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. *Endocrinology*, *150*(11), 4999-5009. https://doi.org/10.1210/en.2009-0500

Dutta, P., Courties, G., Wei, Y., Leuschner, F., Gorbatov, R., Robbins, C. S., Iwamoto, Y., Thompson, B., Carlson, A. L., Heidt, T., Majmudar, M. D., Lasitschka, F., Etzrodt, M., Waterman, P., Waring, M. T., Chicoine, A. T., van der Laan, A. M., Niessen, H. W., Piek, J. J., . . . Nahrendorf, M. (2012). Myocardial infarction accelerates atherosclerosis. *Nature*, *487*(7407), 325-329. https://doi.org/10.1038/nature11260

Eads, C. A., Nickel, A. E., & Laird, P. W. (2002). Complete genetic suppression of polyp formation and reduction of CpG-island hypermethylation in Apc Min/+ Dnmt1-hypomorphic mice. *Cancer research*, *62*(5), 1296-1299.

Edwards, J. R., Yarychkivska, O., Boulard, M., & Bestor, T. H. (2017). DNA methylation and DNA methyltransferases. *Epigenetics & chromatin*, *10*(1), 1-10.

Elliott, B., & Jasin, M. (2002). Double-strand breaks and translocations in cancer. *Cell Mol Life Sci*, *59*(2), 373-385. https://doi.org/10.1007/s00018-002-8429-3

Essex, M. J., Klein, M. H., Cho, E., & Kalin, N. H. (2002). Maternal stress beginning in infancy may sensitize children to later stress exposure: effects on cortisol and behavior. *Biological psychiatry*, *52*(8), 776-784.

Fabian, M. R., & Sonenberg, N. (2012). The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nat Struct Mol Biol*, *19*(6), 586-593. https://doi.org/10.1038/nsmb.2296

Falk, E. (2006). Pathogenesis of atherosclerosis. *Journal of the American College of cardiology*, *47*(8S), C7-C12.

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol*, *1282*, 1-23. https://doi.org/10.1007/978-1-4939-2438-7_1

Feil, R., & Fraga, M. F. (2012). Epigenetics and the environment: emerging patterns and implications. *Nat Rev Genet*, *13*(2), 97-109. https://doi.org/10.1038/nrg3142

Fichorova, R. N., Beatty, N., Sassi, R. R., Yamamoto, H. S., Allred, E. N., Leviton, A., & ELGAN investigators. (2015). Systemic inflammation in the extremely low gestational age newborn following maternal genitourinary infections. *American Journal of Reproductive Immunology*, *73*(2), 162-174.

Fisher, R. A. (1928). Statistical methods for research workers (No. 5). Oliver and Boyd.

Fong, G., Gayen Nee' Betal, S., Murthy, S., Favara, M., Chan, J. S. Y., Addya, S., Shaffer, T. H., Greenspan, J., Bhandari, V., Li, D., Rahman, I., & Aghai, Z. H. (2020). DNA methylation profile in human cord blood mononuclear leukocytes from term neonates: effects of histological chorioamnionitis. *Front Pediatr*, *8*, 437. https://doi.org/10.3389/fped.2020.00437

Frederick, I. O., Rudra, C. B., Miller, R. S., Foster, J. C., & Williams, M. A. (2006). Adult weight change, weight cycling, and prepregnancy obesity in relation to risk of preeclampsia. *Epidemiology*, *17*(4), 428-434. https://doi.org/10.1097/01.ede.0000221028.33245.0b

Friedman, J. H. (1997). On Bias, Variance, 0/1—Loss, and the curse-of-dimensionality. *Data Mining and Knowledge Discovery*, *1*(1), 55-77. https://doi.org/10.1023/A:1009778005914

Gautier, J.-F., Wilson, C., Weyer, C., Mott, D., Knowler, W. C., Cavaghan, M., Polonsky, K. S., Bogardus, C., & Pratley, R. E. (2001). Low acute insulin secretory responses in adult offspring of people with early onset type 2 diabetes. *Diabetes*, *50*(8), 1828-1833.

Gee, S., Chandiramani, M., Seow, J., Pollock, E., Modestini, C., Das, A., Tree, T., Doores, K. J., Tribe, R. M., & Gibbons, D. L. (2021). The legacy of maternal SARS-CoV-2 infection on the immunology of the neonate. *Nat Immunol*, *22*(12), 1490-1502. https://doi.org/10.1038/s41590-021-01049-2

Glass, C. K., & Witztum, J. L. (2001). Atherosclerosis. the road ahead. *Cell*, *104*(4), 503-516. https://doi.org/10.1016/s0092-8674(01)00238-0

Glowacka, I., Bertram, S., Müller, M. A., Allen, P., Soilleux, E., Pfefferle, S., Steffen, I., Tsegaye, T. S., He, Y., Gnirss, K., Niemeyer, D., Schneider, H., Drosten, C., & Pöhlmann, S. (2011). Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol*, *85*(9), 4122-4134. https://doi.org/10.1128/jvi.02232-10

Gluckman, P. D., Hanson, M. A., Buklijas, T., Low, F. M., & Beedle, A. S. (2009). Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nat Rev Endocrinol*, *5*(7), 401-408. https://doi.org/10.1038/nrendo.2009.102

Gluckman, P. D., Hanson, M. A., & Mitchell, M. D. (2010). Developmental origins of health and disease: reducing the burden of chronic disease in the next generation. *Genome Med*, *2*(2), 14. https://doi.org/10.1186/gm135

Gluckman, P. D., Lillycrop, K. A., Vickers, M. H., Pleasants, A. B., Phillips, E. S., Beedle, A. S., Burdge, G. C., & Hanson, M. A. (2007). Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc Natl Acad Sci U S A*, *104*(31), 12796-12800. https://doi.org/10.1073/pnas.0705667104

Godfrey, K. M., Sheppard, A., Gluckman, P. D., Lillycrop, K. A., Burdge, G. C., McLean, C., Rodford, J., Slater-Jefferies, J. L., Garratt, E., Crozier, S. R., Emerald, B. S., Gale, C. R., Inskip, H. M., Cooper, C., & Hanson, M. A. (2011). Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes*, *60*(5), 1528-1534. https://doi.org/10.2337/db10-0979

Goeman, J. J., & Bühlmann, P. (2007). Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics*, *23*(8), 980-987. https://doi.org/10.1093/bioinformatics/btm051

Groß, R., Conzelmann, C., Müller, J. A., Stenger, S., Steinhart, K., Kirchhoff, F., & Münch, J. (2020). Detection of SARS-CoV-2 in human breastmilk. *Lancet*, *395*(10239), 1757-1758. https://doi.org/10.1016/s0140-6736(20)31181-8

Grundy, S. M. (2005). Metabolic syndrome scientific statement by the American Heart Association and the National Heart, Lung, and Blood Institute. *Arterioscler Thromb Vasc Biol*, *25*(11), 2243-2244. https://doi.org/10.1161/01.ATV.0000189155.75833.c7

Guan, L., & Miao, P. (2020). The effects of taurine supplementation on obesity, blood pressure and lipid profile: A meta-analysis of randomized controlled trials. *European journal of pharmacology*, *885*, 173533.

Guarnaccia, A. D., & Tansey, W. P. (2018). Moonlighting with WDR5: a cellular multitasker. *J Clin Med*, *7*(2). https://doi.org/10.3390/jcm7020021

Gudsnuk, K., & Champagne, F. A. (2012). Epigenetic influence of stress and the social environment. *Ilar j*, *53*(3-4), 279-288. https://doi.org/10.1093/ilar.53.3-4.279

Guo, Y. R., Cao, Q. D., Hong, Z. S., Tan, Y. Y., Chen, S. D., Jin, H. J., Tan, K. S., Wang, D. Y., & Yan, Y. (2020). The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res*, *7*(1), 11. https://doi.org/10.1186/s40779-020-00240-0

Ha, K., Lee, G. E., Palii, S. S., Brown, K. D., Takeda, Y., Liu, K., Bhalla, K. N., & Robertson, K. D. (2011). Rapid and transient recruitment of DNMT1 to DNA double-strand breaks is mediated by its interaction with multiple components of the DNA damage response machinery. *Human molecular genetics*, *20*(1), 126-140.

Habas, K., Nganwuchu, C., Shahzad, F., Gopalan, R., Haque, M., Rahman, S., Majumder, A. A., & Nasim, T. (2020). Resolution of coronavirus disease 2019 (COVID-19). *Expert Rev Anti Infect Ther*, *18*(12), 1201-1211. https://doi.org/10.1080/14787210.2020.1797487

Haertle, L., El Hajj, N., Dittrich, M., Müller, T., Nanda, I., Lehnen, H., & Haaf, T. (2017). Epigenetic signatures of gestational diabetes mellitus on cord blood methylation. *Clinical epigenetics*, *9*(1), 1-11.

Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine*, *352*(16), 1685-1695. https://doi.org/10.1056/NEJMra043430

Hara, A., & Taketomi, T. (1991). Characterization and changes of glycosphingolipids in the aorta of the Watanabe hereditable hyperlipidemic rabbit. The Journal of Biochemistry, 109(6), 904-908.

Harrison, A. G., Lin, T., & Wang, P. (2020). Mechanisms of SARS-CoV-2 transmission and pathogenesis. *Trends Immunol*, *41*(12), 1100-1115. https://doi.org/10.1016/j.it.2020.10.004

Harvey, L., & Boksa, P. (2012). Prenatal and postnatal animal models of immune activation: relevance to a range of neurodevelopmental disorders. *Dev Neurobiol*, *72*(10), 1335-1348. https://doi.org/10.1002/dneu.22043

Harvey, Z. H., Chen, Y., & Jarosz, D. F. (2018). Protein-based inheritance: epigenetics beyond the chromosome. *Mol Cell*, *69*(2), 195-202. https://doi.org/10.1016/j.molcel.2017.10.030

Heard, E., & Martienssen, R. A. (2014). Transgenerational epigenetic inheritance: myths and mechanisms. *Cell*, *157*(1), 95-109. https://doi.org/10.1016/j.cell.2014.02.045

Heijmans, B. T., Tobi, E. W., Stein, A. D., Putter, H., Blauw, G. J., Susser, E. S., Slagboom, P. E., & Lumey, L. H. (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A*, *105*(44), 17046-17049. https://doi.org/10.1073/pnas.0806560105

Hill, R. A., Gibbons, A., Han, U., Suwakulsiri, W., Taseska, A., Hammet, F., Southey, M., Malhotra, A., Fahey, M., Palmer, K. R., Hunt, R. W., Lim, I., Newman-Morris, V., & Sundram, S. (2023). Maternal SARS-CoV-2 exposure alters infant DNA methylation. *Brain Behav Immun Health*, *27*, 100572. https://doi.org/10.1016/j.bbih.2022.100572

Hjort, L., Martino, D., Grunnet, L. G., Naeem, H., Maksimovic, J., Olsson, A. H., Zhang, C., Ling, C., Olsen, S. F., & Saffery, R. (2018). Gestational diabetes and maternal obesity are associated with epigenome-wide methylation changes in children. *JCI insight*, *3*(17).

Hoang, N.-M., & Rui, L. (2020). DNA methyltransferases in hematological malignancies. *Journal of Genetics and Genomics*, *47*(7), 361-372.

Hodge, D. R., Cho, E., Copeland, T. D., Guszczynski, T., Yang, E., Seth, A. K., & Farrar, W. L. (2007). IL-6 enhances the nuclear translocation of DNA cytosine-5-methyltransferase 1

(DNMT1) via phosphorylation of the nuclear localization sequence by the AKT kinase. *Cancer Genomics Proteomics*, *4*(6), 387-398.

Hoffmann, M., Kleine-Weber, H., & Pöhlmann, S. (2020). A Multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol Cell*, *78*(4), 779-784.e775. https://doi.org/10.1016/j.molcel.2020.04.022

Holoch, D., & Moazed, D. (2015). RNA-mediated epigenetic regulation of gene expression. *Nat Rev Genet*, *16*(2), 71-84. https://doi.org/10.1038/nrg3863

Hompes, T., Izzi, B., Gellens, E., Morreels, M., Fieuws, S., Pexsters, A., Schops, G., Dom, M., Van Bree, R., Freson, K., Verhaeghe, J., Spitz, B., Demyttenaere, K., Glover, V., Van den Bergh, B., Allegaert, K., & Claes, S. (2013). Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. *J Psychiatr Res*, *47*(7), 880-891. https://doi.org/10.1016/j.jpsychires.2013.03.009

Houshmand-Oeregaard, A., Schrölkamp, M., Kelstrup, L., Hansen, N. S., Hjort, L., Thuesen, A. C. B., Broholm, C., Mathiesen, E. R., Clausen, T. D., Vaag, A., & Damm, P. (2018). Increased expression of microRNA-15a and microRNA-15b in skeletal muscle from adult offspring of women with diabetes in pregnancy. *Human molecular genetics*, *27*(10), 1763-1771. https://doi.org/10.1093/hmg/ddy085

Houtkamp, M. A., de Boer, O. J., van der Loos, C. M., van der Wal, A. C., & Becker, A. E. (2001). Adventitial infiltrates associated with advanced atherosclerotic plaques: structural organization suggests generation of local humoral immune responses. *J Pathol*, *193*(2), 263-269. https://doi.org/10.1002/1096-9896(2000)9999:9999<::Aid-path774>3.0.Co;2-n

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., Codelli, J. A., Chow, J., Reisman, S. E., Petrosino, J. F., Patterson, P. H., & Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, *155*(7), 1451-1463. https://doi.org/10.1016/j.cell.2013.11.024

Hu, Z., Zhou, J., Jiang, J., Yuan, J., Zhang, Y., Wei, X., Loo, N., Wang, Y., Pan, Y., & Zhang, T. (2019). Genomic characterization of genes encoding histone acetylation modulator proteins identifies therapeutic targets for cancer treatment. *Nature communications*, *10*(1), 733.

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic acids research*, *37*(1), 1-13.

Huang, I. C., Bosch, B. J., Li, F., Li, W., Lee, K. H., Ghiran, S., Vasilieva, N., Dermody, T. S., Harrison, S. C., Dormitzer, P. R., Farzan, M., Rottier, P. J., & Choe, H. (2006). SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. *J Biol Chem*, *281*(6), 3198-3203. https://doi.org/10.1074/jbc.M508381200

Hui, D. S., E, I. A., Madani, T. A., Ntoumi, F., Kock, R., Dar, O., Ippolito, G., McHugh, T. D., Memish, Z. A., Drosten, C., Zumla, A., & Petersen, E. (2020). The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis*, *91*, 264-266. https://doi.org/10.1016/j.ijid.2020.01.009

Huypens, P., Sass, S., Wu, M., Dyckhoff, D., Tschöp, M., Theis, F., Marschall, S., Hrabě de Angelis, M., & Beckers, J. (2016). Epigenetic germline inheritance of diet-induced obesity and insulin resistance. *Nat Genet*, *48*(5), 497-499. https://doi.org/10.1038/ng.3527

Ito, T., Yoshikawa, N., Inui, T., Miyazaki, N., Schaffer, S. W., & Azuma, J. (2014). Tissue depletion of taurine accelerates skeletal muscle senescence and leads to early death in mice. *PloS one*, *9*(9), e107409.

Izzo, A., & Schneider, R. (2016). The role of linker histone H1 modifications in the regulation of gene expression and chromatin dynamics. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1859*(3), 486-495.

Jackson, C. B., Farzan, M., Chen, B., & Choe, H. (2022). Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol Cell Biol*, *23*(1), 3-20. https://doi.org/10.1038/s41580-021-00418-x

Jaenisch, R., & Bird, A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet*, *33 Suppl*, 245-254. https://doi.org/10.1038/ng1089

Jeganathan, K., & Paul, A. B. (2022). Vertical transmission of SARS-CoV-2: a systematic review. *Obstetric Medicine*, *15*(2), 91-98.

Ji, H., Yuan, L., Jiang, W., Jiang, Y., Jiang, M., Sun, X., & Chen, J. (2023). Bioinformatics analysis of immune cell infiltration patterns and potential diagnostic markers in atherosclerosis. *Sci Rep*, *13*(1), 19821. https://doi.org/10.1038/s41598-023-47257-8

Jiang, H. Y., Xu, L. L., Shao, L., Xia, R. M., Yu, Z. H., Ling, Z. X., Yang, F., Deng, M., & Ruan, B. (2016). Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. *Brain Behav Immun*, *58*, 165-172. https://doi.org/10.1016/j.bbi.2016.06.005

Jin, Y. H., Cai, L., Cheng, Z. S., Cheng, H., Deng, T., Fan, Y. P., Fang, C., Huang, D., Huang, L. Q., Huang, Q., Han, Y., Hu, B., Hu, F., Li, B. H., Li, Y. R., Liang, K., Lin, L. K., Luo, L. S., Ma, J., . . . Wang, X. H. (2020). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil Med Res*, *7*(1), 4. https://doi.org/10.1186/s40779-020-0233-6

Joubert, B. R., den Dekker, H. T., Felix, J. F., Bohlin, J., Ligthart, S., Beckett, E., Tiemeier, H., van Meurs, J. B., Uitterlinden, A. G., Hofman, A., Håberg, S. E., Reese, S. E., Peters, M. J., Andreassen, B. K., Steegers, E. A., Nilsen, R. M., Vollset, S. E., Midttun, Ø., Ueland, P. M., . . . London, S. J. (2016). Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. *Nat Commun*, *7*, 10577. https://doi.org/10.1038/ncomms10577

Kahn, J. S., & McIntosh, K. (2005). History and recent advances in coronavirus discovery. *Pediatr Infect Dis J*, *24*(11 Suppl), S223-227, discussion S226. https://doi.org/10.1097/01.inf.0000188166.17324.60

Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., & Tanabe, M. (2016). KEGG as a reference resource for gene and protein annotation. *Nucleic acids research*, *44*(D1), D457-D462.

Kang, J., Lee, C.-N., Li, H.-Y., Hsu, K.-H., & Lin, S.-Y. (2017). Genome-wide DNA methylation variation in maternal and cord blood of gestational diabetes population. *Diabetes Research and Clinical Practice*, *132*, 127-136.

Kim, C., & Cha, Y. N. (2014). Taurine chloramine produced from taurine under inflammation provides anti-inflammatory and cytoprotective effects. *Amino acids*, *46*(1), 89-100. https://doi.org/10.1007/s00726-013-1545-6

Kimberlin, D. W., & Puopolo, K. M. (2021). Breast milk and COVID-19: what do we know? *Clin Infect Dis*, *72*(1), 131-132. https://doi.org/10.1093/cid/ciaa800

Kleeman, E. A., Gubert, C., & Hannan, A. J. (2022). Transgenerational epigenetic impacts of parental infection on offspring health and disease susceptibility. *Trends Genet*, *38*(7), 662-675. https://doi.org/10.1016/j.tig.2022.03.006

Knobler, S., Mahmoud, A., Lemon, S., Mack, A., Sivitz, L., & Oberholtzer, K. (2004). In the Absence of SARS-CoV Transmission Worldwide: Guidance for Surveillance, Clinical and Laboratory Evaluation, and Reporting. In Learning from SARS: Preparing for the Next Disease Outbreak: Workshop Summary. *National Academies Press (US)*.

Kocher, K., Bhattacharya, S., Niforatos-Andescavage, N., Almalvez, M., Henderson, D., Vilain, E., Limperopoulos, C., & Délot, E. C. (2023). Genome-wide neonatal epigenetic changes associated with maternal exposure to the COVID-19 pandemic. *BMC Med Genomics*, *16*(1), 268. https://doi.org/10.1186/s12920-023-01707-4

Kotsakis Ruehlmann, A., Sammallahti, S., Cortés Hidalgo, A. P., Bakulski, K. M., Binder, E. B., Campbell, M. L., Caramaschi, D., Cecil, C. A. M., Colicino, E., Cruceanu, C., Czamara, D., Dieckmann, L., Dou, J., Felix, J. F., Frank, J., Håberg, S. E., Herberth, G., Hoang, T. T., Houtepen, L. C., . . . Brunst, K. J. (2023). Epigenome-wide meta-analysis of prenatal maternal stressful life events and newborn DNA methylation. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-023-02010-5

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*(4), 693-705.

Kratsman, N., Getselter, D., & Elliott, E. (2016). Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model. *Neuropharmacology*, *102*, 136-145. https://doi.org/10.1016/j.neuropharm.2015.11.003

Labouesse, M. A., Dong, E., Grayson, D. R., Guidotti, A., & Meyer, U. (2015). Maternal immune activation induces GAD1 and GAD2 promoter remodeling in the offspring prefrontal cortex. *Epigenetics*, *10*(12), 1143-1155.

Laderoute, M. P. (2015). A new paradigm about HERV-K102 particle production and blocked release to explain cortisol mediated immunosenescence and age-associated risk of chronic disease. *Discov Med*, *20*(112), 379-391.

Laderoute, M. P. (2020). The paradigm of immunosenescence in atherosclerosis-cardiovascular disease (ASCVD). *Discovery Medicine*, *29*(156), 41-51.

Lai, S., Hua, X., Gao, R., Zeng, L., Song, J., Liu, J., & Zhang, J. (2018). Combinational biomarkers for atrial fibrillation derived from atrial appendage and plasma metabolomics analysis. *Scientific Reports*, *8*(1), 16930. https://doi.org/10.1038/s41598-018-34930-6

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., & Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, *581*(7807), 215-220. https://doi.org/10.1038/s41586-020-2180-5

Lawrence, M., Daujat, S., & Schneider, R. (2016). Lateral thinking: how histone modifications regulate gene expression. *Trends in Genetics*, *32*(1), 42-56.

Lester, B. M., Conradt, E., & Marsit, C. J. (2013). Epigenetic basis for the development of depression in children. *Clin Obstet Gynecol*, *56*(3), 556-565. https://doi.org/10.1097/GRF.0b013e318299d2a8

Leviton, A., Allred, E. N., Kuban, K. C., O'Shea, T. M., Paneth, N., Onderdonk, A. B., ... & Dammann, O. (2016). The development of extremely preterm infants born to women who had genitourinary infections during pregnancy. *American journal of epidemiology*, *183*(1), 28-35.

Levy, A., Yagil, Y., Bursztyn, M., Barkalifa, R., Scharf, S., & Yagil, C. (2008). ACE2 expression and activity are enhanced during pregnancy. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(6), R1953-R1961.

Lewsey, M. G., Hardcastle, T. J., Melnyk, C. W., Molnar, A., Valli, A., Urich, M. A., Nery, J. R., Baulcombe, D. C., & Ecker, J. R. (2016). Mobile small RNAs regulate genome-wide DNA methylation. *Proceedings of the National Academy of Sciences*, *113*(6), E801-E810.

Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., Somasundaran, M., Sullivan, J. L., Luzuriaga, K., Greenough, T. C., Choe, H., & Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*, *426*(6965), 450-454. https://doi.org/10.1038/nature02145

Li, W., Zhang, C., Sui, J., Kuhn, J. H., Moore, M. J., Luo, S., Wong, S. K., Huang, I. C., Xu, K., Vasilieva, N., Murakami, A., He, Y., Marasco, W. A., Guan, Y., Choe, H., & Farzan, M. (2005). Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. *Embo j*, *24*(8), 1634-1643. https://doi.org/10.1038/sj.emboj.7600640

Li, Y., Zhao, R., Zheng, S., Chen, X., Wang, J., Sheng, X., Zhou, J., Cai, H., Fang, Q., & Yu, F. (2020). Lack of vertical transmission of severe acute respiratory syndrome coronavirus 2, China. *Emerging infectious diseases*, *26*(6), 1335.

Libby, P. (2002). Inflammation in atherosclerosis. *Nature*, *420*(6917), 868-874. https://doi.org/10.1038/nature01323

Limperopoulos, C., Bassan, H., Sullivan, N. R., Soul, J. S., Robertson, R. L., Jr., Moore, M., Ringer, S. A., Volpe, J. J., & du Plessis, A. J. (2008). Positive screening for autism in ex-preterm infants: prevalence and risk factors. *Pediatrics*, *121*(4), 758-765. https://doi.org/10.1542/peds.2007-2158

Liu, L., Liu, Y., Liu, C., Zhang, Z., Du, Y., & Zhao, H. (2016). Analysis of gene expression profile identifies potential biomarkers for atherosclerosis. *Molecular medicine reports*, *14*(4), 3052-3058.

Lo, T. C., Cheng, J. Y., Lee, C. W., Hung, J. T., Lin, C. C., Yeh, H. F., Yang, B. C., Huang, Y., Wu, H. M., Yu, A. L., & Yu, J. (2023). Priming of macrophage by glycosphingolipids from extracellular vesicles facilitates immune tolerance for embryo-maternal crosstalk. *Dev Cell*, *58*(22), 2447-2459.e2445. https://doi.org/10.1016/j.devcel.2023.09.012

Lu, H., Chen, Y., & Li, L. (2018). Metabolic pathway genes associated with susceptibility genes to coronary artery disease. *International journal of genomics*, *2018*.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., . . . Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*, *395*(10224), 565-574. https://doi.org/10.1016/s0140-6736(20)30251-8

Lyko, F. (2018). The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. *Nature Reviews Genetics*, *19*(2), 81-92.

Machón, R. A., Mednick, S. A., & Huttunen, M. O. (1997). Adult major affective disorder after prenatal exposure to an influenza epidemic. *Archives of General Psychiatry*, *54*(4), 322-328. https://doi.org/10.1001/archpsyc.1997.01830160040006

Mackay, I. M., & Arden, K. E. (2015). MERS coronavirus: diagnostics, epidemiology and transmission. *Virol J*, *12*, 222. https://doi.org/10.1186/s12985-015-0439-5

Mahmoudi, M., Mercer, J., & Bennett, M. (2006). DNA damage and repair in atherosclerosis. *Cardiovascular Research*, *71*(2), 259-268. https://doi.org/10.1016/j.cardiores.2006.03.002

Malakar, A. K., Choudhury, D., Halder, B., Paul, P., Uddin, A., & Chakraborty, S. (2019). A review on coronary artery disease, its risk factors, and therapeutics. *Journal of cellular physiology*, *234*(10), 16812-16823.

Maleki, F., Ovens, K., Hogan, D. J., & Kusalik, A. J. (2020). Gene set analysis: challenges, opportunities, and future research [review]. *Frontiers in Genetics*, *11*. https://doi.org/10.3389/fgene.2020.00654

Malik, Y. A. (2020). Properties of Coronavirus and SARS-CoV-2. *Malays J Pathol*, *42*(1), 3-11.

Matute, J. D., Finander, B., Pepin, D., Ai, X., Smith, N. P., Li, J. Z., Edlow, A. G., Villani, A.-C., Lerou, P. H., & Kalish, B. T. (2022). Single-cell immunophenotyping of the fetal immune response to maternal SARS-CoV-2 infection in late gestation. *Pediatric Research*, *91*(5), 1090-1098. https://doi.org/10.1038/s41390-021-01793-z

Mazumder, B., Almond, D., Park, K., Crimmins, E. M., & Finch, C. E. (2010). Lingering prenatal effects of the 1918 influenza pandemic on cardiovascular disease. *J Dev Orig Health Dis*, *1*(1), 26-34. https://doi.org/10.1017/s2040174409990031

McCullough, L. E., Miller, E. E., Calderwood, L. E., Shivappa, N., Steck, S. E., Forman, M. R., A. Mendez, M., Maguire, R., Fuemmeler, B. F., & Kollins, S. H. (2017). Maternal inflammatory diet and adverse pregnancy outcomes: Circulating cytokines and genomic imprinting as potential regulators? *Epigenetics*, *12*(8), 688-697.

Mednick, S. A., Machon, R. A., Huttunen, M. O., & Bonett, D. (1988). Adult schizophrenia following prenatal exposure to an influenza epidemic. *Archives of General Psychiatry*, *45*(2), 189-192. https://doi.org/10.1001/archpsyc.1988.01800260109013

Michels, K. B., Harris, H. R., & Barault, L. (2011). Birthweight, maternal weight trajectories and global DNA methylation of LINE-1 repetitive elements. *PloS one*, *6*(9), e25254.

Mirbeyk, M., Saghazadeh, A., & Rezaei, N. (2021). A systematic review of pregnant women with COVID-19 and their neonates. *Arch Gynecol Obstet*, *304*(1), 5-38. https://doi.org/10.1007/s00404-021-06049-z

Mitrea, C., Taghavi, Z., Bokanizad, B., Hanoudi, S., Tagett, R., Donato, M., Voichiţa, C., & Drăghici, S. (2013). Methods and approaches in the topology-based analysis of biological pathways. *Frontiers in physiology*, *4*, 278.

Moreno-Fernandez, J., Ochoa, J. J., De Paco Matallana, C., Caño, A., Martín-Alvarez, E., Sanchez-Romero, J., Toledano, J. M., Puche-Juarez, M., Prados, S., & Ruiz-Duran, S. (2022). COVID-19 during gestation: maternal implications of evoked oxidative stress and iron metabolism impairment. *Antioxidants*, *11*(2), 184.

Moutinho, C., & Esteller, M. (2017). MicroRNAs and epigenetics. *Adv Cancer Res*, *135*, 189-220. https://doi.org/10.1016/bs.acr.2017.06.003

MSigDB, R. *Human Gene Set: REACTOME_EPIGENETIC_REGULATION_OF_GENE_EXPRESSION*. https://www.gsea-msigdb.org/gsea/msigdb/cards/REACTOME_EPIGENETIC_REGULATION_OF_GENE_EXPRESSION

Müller, L., Di Benedetto, S., & Pawelec, G. (2019). The immune system and its dysregulation with aging. *Subcell Biochem*, *91*, 21-43. https://doi.org/10.1007/978-981-13-3681-2_2

Mulligan, C. J., D'Errico, N. C., Stees, J., & Hughes, D. A. (2012). Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. *Epigenetics*, *7*(8), 853-857. https://doi.org/10.4161/epi.21180

Murphy, S. (2020). Newborn baby tests positive for coronavirus in London. *The Guardian*. Retrieved 17 February 2024 from https://www.theguardian.com/world/2020/mar/14/newborn-baby-tests-positive-for-coronavirus-in-london

Musa, S. S., Bello, U. M., Zhao, S., Abdullahi, Z. U., Lawan, M. A., & He, D. (2021). Vertical transmission of SARS-CoV-2: a systematic review of systematic reviews. *Viruses*, *13*(9), 1877.

Myrskylä, M., Mehta, N. K., & Chang, V. W. (2013). Early life exposure to the 1918 influenza pandemic and old-age mortality by cause of death. *Am J Public Health*, *103*(7), e83-90. https://doi.org/10.2105/ajph.2012.301060

Nam, D., & Kim, S. Y. (2008). Gene-set approach for expression pattern analysis. *Brief Bioinform*, *9*(3), 189-197. https://doi.org/10.1093/bib/bbn001

Niculescu, M. D., & Zeisel, S. H. (2002). Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. *J Nutr*, *132*(8 Suppl), 2333s-2335s. https://doi.org/10.1093/jn/132.8.2333S

Norberg, S., Powell, T. L., & Jansson, T. (1998). Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters. *Pediatr Res*, *44*(2), 233-238. https://doi.org/10.1203/00006450-199808000-00016

Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*, *3*(2), 97-106. https://doi.org/10.4161/epi.3.2.6034

Oishi, Y., & Manabe, I. (2016). Macrophages in age-related chronic inflammatory diseases. *NPJ Aging Mech Dis*, *2*, 16018. https://doi.org/10.1038/npjamd.2016.18

Otter, J. A., Donskey, C., Yezli, S., Douthwaite, S., Goldenberg, S. D., & Weber, D. J. (2016). Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. *J Hosp Infect*, *92*(3), 235-250. https://doi.org/10.1016/j.jhin.2015.08.027

Ozturk, S., & Demir, N. (2011). DNA repair mechanisms in mammalian germ cells. *Histology and histopathology, Vol. 26, nº 4 (2011)*.

Pan, K.-H., Lih, C.-J., & Cohen, S. N. (2005). Effects of threshold choice on biological conclusions reached during analysis of gene expression by DNA microarrays. *Proceedings of the National Academy of Sciences*, *102*(25), 8961-8965.

Parboosing, R., Bao, Y., Shen, L., Schaefer, C. A., & Brown, A. S. (2013). Gestational influenza and bipolar disorder in adult offspring. *JAMA psychiatry*, *70*(7), 677-685. https://doi.org/10.1001/jamapsychiatry.2013.896

Park, Y., & Kuroda, M. I. (2001). Epigenetic aspects of X-chromosome dosage compensation. *Science*, *293*(5532), 1083-1085.

Perła-Kaján, J., & Jakubowski, H. (2022). COVID-19 and one-carbon metabolism. *International Journal of Molecular Sciences*, *23*(8), 4181.

Perlman, S., & Netland, J. (2009). Coronaviruses post-SARS: update on replication and pathogenesis. *Nature Reviews Microbiology*, *7*(6), 439-450. https://doi.org/10.1038/nrmicro2147

Pierce, D. A., Shimizu, Y., Preston, D. L., Vaeth, M., & Mabuchi, K. (1996). Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer: 1950-1990. *Radiat Res*, *146*(1), 1-27.

Pradhan, J., Mallick, S., Mishra, N., Tiwari, A., & Negi, V. D. (2023). Pregnancy, infection, and epigenetic regulation: A complex scenario. *Biochim Biophys Acta Mol Basis Dis*, *1869*(7), 166768. https://doi.org/10.1016/j.bbadis.2023.166768

Preising, M. N., Görg, B., Friedburg, C., Qvartskhava, N., Budde, B. S., Bonus, M., Toliat, M. R., Pfleger, C., Altmüller, J., Herebian, D., Beyer, M., Zöllner, H. J., Wittsack, H. J., Schaper, J., Klee, D., Zechner, U., Nürnberg, P., Schipper, J., Schnitzler, A., . . . Bolz, H. J. (2019). Biallelic mutation of human SLC6A6 encoding the taurine transporter TAUT is linked to early retinal degeneration. *Faseb j*, *33*(10), 11507-11527. https://doi.org/10.1096/fj.201900914RR

Preston, D. L., Kusumi, S., Tomonaga, M., Izumi, S., Ron, E., Kuramoto, A., Kamada, N., Dohy, H., Matsuo, T., Matsui, T., & et al. (1994). Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950-1987. *Radiat Res*, *137*(2 Suppl), S68-97.

Prochaska, E., Jang, M., & Burd, I. (2020). COVID-19 in pregnancy: Placental and neonatal involvement. *Am J Reprod Immunol*, *84*(5), e13306. https://doi.org/10.1111/aji.13306

Quackenbush, J. (2001). Computational analysis of microarray data. *Nature Reviews Genetics*, *2*(6), 418-427.

Rahman, S., Montero, M. T. V., Rowe, K., Kirton, R., & Kunik, F., Jr. (2021). Epidemiology, pathogenesis, clinical presentations, diagnosis and treatment of COVID-19: a review of current evidence. *Expert Rev Clin Pharmacol*, *14*(5), 601-621. https://doi.org/10.1080/17512433.2021.1902303

Rasmussen, S. A., Jamieson, D. J., & Uyeki, T. M. (2012). Effects of influenza on pregnant women and infants. *Am J Obstet Gynecol*, *207*(3 Suppl), S3-8. https://doi.org/10.1016/j.ajog.2012.06.068

Rassool, F. V. (2003). DNA double strand breaks (DSB) and non-homologous end joining (NHEJ) pathways in human leukemia. *Cancer letters*, *193*(1), 1-9.

REACTOME. *REACTOME_EPIGENETIC_REGULATION_OF_GENE_EXPRESSION*. Retrieved Fenruary 20 from https://www.reactome.org/content/detail/R-HSA-212165

Reichetzeder, C., Putra, S., & Pfab, T. (2016). Increased global placental DNA methylation levels are associated with gestational diabetes. Clin Epigenetics 8: 82. In.

Relton, C. L., Groom, A., St. Pourcain, B., Sayers, A. E., Swan, D. C., Embleton, N. D., Pearce, M. S., Ring, S. M., Northstone, K., & Tobias, J. H. (2012). DNA methylation patterns in cord blood DNA and body size in childhood. *PloS one*, *7*(3), e31821.

Ren, W., Gao, L., & Song, J. (2018). Structural basis of DNMT1 and DNMT3A-mediated DNA methylation. *Genes*, *9*(12), 620.

Ricketts, M. D., Han, J., Szurgot, M. R., & Marmorstein, R. (2019). Molecular basis for chromatin assembly and modification by multiprotein complexes. *Protein Science*, *28*(2), 329-343.

Ripps, H., & Shen, W. (2012). Review: taurine: a "very essential" amino acid. *Mol Vis*, *18*, 2673-2686.

Rodríguez-Rodríguez, P., Ramiro-Cortijo, D., Reyes-Hernández, C. G., López de Pablo, A. L., González, M. C., & Arribas, S. M. (2018). Implication of oxidative stress in fetal programming of cardiovascular disease [review]. *Frontiers in physiology*, *9*. https://doi.org/10.3389/fphys.2018.00602

Rolfo, A., Cosma, S., Nuzzo, A. M., Salio, C., Moretti, L., Sassoè-Pognetto, M., Carosso, A. R., Borella, F., Cutrin, J. C., & Benedetto, C. (2022). Increased placental anti-oxidant response

in asymptomatic and symptomatic COVID-19 third-trimester pregnancies. *Biomedicines*, *10*(3), 634.

Roseboom, T. J., van der Meulen, J. H., Ravelli, A. C., Osmond, C., Barker, D. J., & Bleker, O. P. (2001). Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Twin Res*, *4*(5), 293-298. https://doi.org/10.1375/1369052012605

Roysommuti, S., & Wyss, J. M. (2014). Perinatal taurine exposure affects adult arterial pressure control. *Amino acids*, *46*(1), 57-72. https://doi.org/10.1007/s00726-012-1417-5

Ruchat, S. M., Houde, A. A., Voisin, G., St-Pierre, J., Perron, P., Baillargeon, J. P., Gaudet, D., Hivert, M. F., Brisson, D., & Bouchard, L. (2013). Gestational diabetes mellitus epigenetically affects genes predominantly involved in metabolic diseases. *Epigenetics*, *8*(9), 935-943. https://doi.org/10.4161/epi.25578

Rudolph, M. D., Graham, A. M., Feczko, E., Miranda-Dominguez, O., Rasmussen, J. M., Nardos, R., Entringer, S., Wadhwa, P. D., Buss, C., & Fair, D. A. (2018). Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. *Nature Neuroscience*, *21*(5), 765-772. https://doi.org/10.1038/s41593-018-0128-y

Salisbury, A. L., Wisner, K. L., Pearlstein, T., Battle, C. L., Stroud, L., & Lester, B. M. (2011). Newborn neurobehavioral patterns are differentially related to prenatal maternal major depressive disorder and serotonin reuptake inhibitor treatment. *Depression and anxiety*, *28*(11), 1008-1019.

Sandman, C. A., Davis, E. P., Buss, C., & Glynn, L. M. (2011). Prenatal programming of human neurological function. *International journal of peptides*, *2011*.

Santarpia, J. L., Rivera, D. N., Herrera, V., Morwitzer, M. J., Creager, H., Santarpia, G. W., Crown, K. K., Brett-Major, D. M., Schnaubelt, E., & Broadhurst, M. J. (2020). Transmission potential of SARS-CoV-2 in viral shedding observed at the University of Nebraska Medical Center. *MedRxiv*.

Sardar, S. W., Nam, J., Kim, T. E., Kim, H., & Park, Y. H. (2023). Identification of novel biomarkers for early diagnosis of atherosclerosis using high-resolution metabolomics. *Metabolites*, *13*(11), 1160. https://www.mdpi.com/2218-1989/13/11/1160

Shah, R., Murthy, V., Pacold, M., Danielson, K., Tanriverdi, K., Larson, M. G., Hanspers, K., Pico, A., Mick, E., & Reis, J. (2017). Extracellular RNAs are associated with insulin resistance and metabolic phenotypes. *Diabetes care*, *40*(4), 546-553.

Shaknovich, R., Cerchietti, L., Tsikitas, L., Kormaksson, M., De, S., Figueroa, M. E., Ballon, G., Yang, S. N., Weinhold, N., & Reimers, M. (2011). DNA methyltransferase 1 and DNA methylation patterning contribute to germinal center B-cell differentiation. *Blood, The Journal of the American Society of Hematology*, *118*(13), 3559-3569.

Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., & Li, F. (2020). Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A*, *117*(21), 11727-11734. https://doi.org/10.1073/pnas.2003138117

Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., & Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature*, *581*(7807), 221-224. https://doi.org/10.1038/s41586-020-2179-y

Sharma, A., Ahmad Farouk, I., & Lal, S. K. (2021). COVID-19: A Review on the novel coronavirus eisease evolution, transmission, detection, control and prevention. *Viruses*, *13*(2). https://doi.org/10.3390/v13020202

Sharp, G. C., Salas, L. A., Monnereau, C., Allard, C., Yousefi, P., Everson, T. M., Bohlin, J., Xu, Z., Huang, R.-C., & Reese, S. E. (2017). Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation: findings from the pregnancy and childhood epigenetics (PACE) consortium. *Human molecular genetics*, *26*(20), 4067-4085.

Shiau, S., Strehlau, R., Wang, S., Violari, A., Do, C., Patel, F., Liberty, A., Krupska, I., Arpadi, S. M., & Foca, M. (2019). Distinct epigenetic profiles in children with perinatally-acquired HIV on antiretroviral therapy. *Scientific Reports*, *9*(1), 10495.

Shulla, A., Heald-Sargent, T., Subramanya, G., Zhao, J., Perlman, S., & Gallagher, T. (2011). A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. *J Virol*, *85*(2), 873-882. https://doi.org/10.1128/jvi.02062-10

Simmons, G., Gosalia, D. N., Rennekamp, A. J., Reeves, J. D., Diamond, S. L., & Bates, P. (2005). Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proc Natl Acad Sci U S A*, *102*(33), 11876-11881. https://doi.org/10.1073/pnas.0505577102

Simpkin, A. J., Suderman, M., Gaunt, T. R., Lyttleton, O., McArdle, W. L., Ring, S. M., Tilling, K., Davey Smith, G., & Relton, C. L. (2015). Longitudinal analysis of DNA methylation associated with birth weight and gestational age. *Human molecular genetics*, *24*(13), 3752-3763.

Singh, P., Gollapalli, K., Mangiola, S., Schranner, D., Yusuf, M. A., Chamoli, M., Shi, S. L., Lopes Bastos, B., Nair, T., Riermeier, A., Vayndorf, E. M., Wu, J. Z., Nilakhe, A., Nguyen, C. Q., Muir, M., Kiflezghi, M. G., Foulger, A., Junker, A., Devine, J., . . . Yadav, V. K. (2023). Taurine deficiency as a driver of aging. *Science*, *380*(6649), eabn9257. https://doi.org/10.1126/science.abn9257

Snijder, E. J., van der Meer, Y., Zevenhoven-Dobbe, J., Onderwater, J. J., van der Meulen, J., Koerten, H. K., & Mommaas, A. M. (2006). Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. *J Virol*, *80*(12), 5927-5940. https://doi.org/10.1128/jvi.02501-05

Solomons, N. W. (2009). Developmental origins of health and disease: concepts, caveats, and consequences for public health nutrition. *Nutr Rev*, *67 Suppl 1*, S12-16. https://doi.org/10.1111/j.1753-4887.2009.00152.x

Song, W., Gui, M., Wang, X., & Xiang, Y. (2018). Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. *PLoS Pathog*, *14*(8), e1007236. https://doi.org/10.1371/journal.ppat.1007236

Sorci, G., Faivre, B., & Morand, S. (2020). Explaining among-country variation in COVID-19 case fatality rate. *Scientific Reports*, *10*(1), 18909. https://doi.org/10.1038/s41598-020-75848-2

Spagnoli, L. G., Bonanno, E., Sangiorgi, G., & Mauriello, A. (2007). Role of inflammation in atherosclerosis. *Journal of Nuclear Medicine*, *48*(11), 1800-1815. https://doi.org/10.2967/jnumed.107.038661

The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. (2020). *Nat Microbiol*, *5*(4), 536-544. https://doi.org/10.1038/s41564-020-0695-z

Stelzer, I. A., Urbschat, C., Schepanski, S., Thiele, K., Triviai, I., Wieczorek, A., ... & Arck, P. C. (2021). Vertically transferred maternal immune cells promote neonatal immunity against early life infections. *Nature Communications*, 12(1), 4706.

Strakovsky, R. S., Zhang, X., Zhou, D., & Pan, Y. X. (2014). The regulation of hepatic Pon1 by a maternal high-fat diet is gender specific and may occur through promoter histone modifications in neonatal rats. *J Nutr Biochem*, *25*(2), 170-176. https://doi.org/10.1016/j.jnutbio.2013.09.016

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. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, *102*(43), 15545-15550.

Sumathipala, D., Gamage, T., Wijesiriwardena, B., Jayasekara, R. W., & Dissanayake, V. H. (2012). An infant born to a mother with gestational diabetes presenting with 49,XXXXY syndrome and renal agenesis-a case report. *J Clin Res Pediatr Endocrinol*, *4*(4), 223-225. https://doi.org/10.4274/jcrpe.764

Sun, W., Dong, H., Becker, A. S., Dapito, D. H., Modica, S., Grandl, G., Opitz, L., Efthymiou, V., Straub, L. G., Sarker, G., Balaz, M., Balazova, L., Perdikari, A., Kiehlmann, E., Bacanovic, S., Zellweger, C., Peleg-Raibstein, D., Pelczar, P., Reik, W., . . . Wolfrum, C. (2018). Cold-induced epigenetic programming of the sperm enhances brown adipose tissue activity in the offspring. *Nat Med*, *24*(9), 1372-1383. https://doi.org/10.1038/s41591-018-0102-y

Suter, M., Bocock, P., Showalter, L., Hu, M., Shope, C., McKnight, R., Grove, K., Lane, R., & Aagaard-Tillery, K. (2011). Epigenomics: maternal high-fat diet exposure in utero disrupts peripheral circadian gene expression in nonhuman primates. *Faseb j*, *25*(2), 714-726. https://doi.org/10.1096/fj.10-172080

Swirski, F. K., Libby, P., Aikawa, E., Alcaide, P., Luscinskas, F. W., Weissleder, R., & Pittet, M. J. (2007). Ly-6C hi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. The Journal of clinical investigation, 117(1), 195-205.

Szyf, M. (2015). Prospects for the development of epigenetic drugs for CNS conditions. *Nat Rev Drug Discov*, *14*(7), 461-474. https://doi.org/10.1038/nrd4580

Tamashiro, K. L., & Moran, T. H. (2010). Perinatal environment and its influences on metabolic programming of offspring. *Physiol Behav*, *100*(5), 560-566. https://doi.org/10.1016/j.physbeh.2010.04.008

Tang, W. W., Kobayashi, T., Irie, N., Dietmann, S., & Surani, M. A. (2016). Specification and epigenetic programming of the human germ line. *Nat Rev Genet*, *17*(10), 585-600. https://doi.org/10.1038/nrg.2016.88

Tannetta, D., Dragovic, R., Alyahyaei, Z., & Southcombe, J. (2014). Extracellular vesicles and reproduction–promotion of successful pregnancy. *Cellular & Molecular Immunology*, *11*(6), 548-563. https://doi.org/10.1038/cmi.2014.42

Team, R. D. C. (2009). *A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.* https://www.r-project.org/

Tepebaşı, M. Y., İlhan, İ., Temal, E. N., Sancer, O., & Öztürk, Ö. (2022). The effect of inflammation and oxidative stress on DNA damage in COVID-19 patients.

Thomas, T., Stefanoni, D., Reisz, J. A., Nemkov, T., Bertolone, L., Francis, R. O., Hudson, K. E., Zimring, J. C., Hansen, K. C., & Hod, E. A. (2020). COVID-19 infection alters kynurenine and fatty acid metabolism, correlating with IL-6 levels and renal status. *JCI insight*, *5*(14).

Tian, L., Greenberg, S. A., Kong, S. W., Altschuler, J., Kohane, I. S., & Park, P. J. (2005). Discovering statistically significant pathways in expression profiling studies. Proceedings

of the National Academy of Sciences of the United States of America, 102(38), 13544–13549. https://doi.org/10.1073/pnas.0506577102.

Tobi, E. W., Lumey, L. H., Talens, R. P., Kremer, D., Putter, H., Stein, A. D., Slagboom, P. E., & Heijmans, B. T. (2009). DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet*, *18*(21), 4046-4053. https://doi.org/10.1093/hmg/ddp353

Tsai, C. A., & Chen, J. J. (2009). Multivariate analysis of variance test for gene set analysis. *Bioinformatics*, *25*(7), 897-903. https://doi.org/10.1093/bioinformatics/btp098

Tyrrell, D. A., & Bynoe, M. L. (1966). Cultivation of viruses from a high proportion of patients with colds. *Lancet*, *1*(7428), 76-77. https://doi.org/10.1016/s0140-6736(66)92364-6

Urday, P., Gayen Nee' Betal, S., Sequeira Gomes, R., Al-Kouatly, H. B., Solarin, K., Chan, J. S., Li, D., Rahman, I., Addya, S., Boelig, R. C., & Aghai, Z. H. (2023). SARS-CoV-2 covid-19 infection during pregnancy and differential DNA methylation in human cord blood cells from term neonates. *Epigenet Insights*, *16*, 25168657231184665. https://doi.org/10.1177/25168657231184665

van der Hoek, L., Pyrc, K., Jebbink, M. F., Vermeulen-Oost, W., Berkhout, R. J., Wolthers, K. C., Wertheim-van Dillen, P. M., Kaandorp, J., Spaargaren, J., & Berkhout, B. (2004). Identification of a new human coronavirus. *Nat Med*, *10*(4), 368-373. https://doi.org/10.1038/nm1024

Van Dijk, S., Peters, T., Buckley, M., Zhou, J., Jones, P., Gibson, R., Makrides, M., Muhlhausler, B., & Molloy, P. (2018). DNA methylation in blood from neonatal screening cards and the association with BMI and insulin sensitivity in early childhood. *International journal of obesity*, *42*(1), 28-35.

Veland, N., Lu, Y., Hardikar, S., Gaddis, S., Zeng, Y., Liu, B., Estecio, M. R., Takata, Y., Lin, K., & Tomida, M. W. (2019). DNMT3L facilitates DNA methylation partly by maintaining DNMT3A stability in mouse embryonic stem cells. *Nucleic acids research*, *47*(1), 152-167.

Verschuren, J. J., Trompet, S., Deelen, J., Stott, D. J., Sattar, N., Buckley, B. M., Ford, I., Heijmans, B. T., Guchelaar, H. J., Houwing-Duistermaat, J. J., Slagboom, P. E., & Jukema, J. W. (2013). Non-homologous end-joining pathway associated with occurrence of myocardial infarction: gene set analysis of genome-wide association study data. *PloS one*, *8*(2), e56262. https://doi.org/10.1371/journal.pone.0056262

Vogel, T., & Lassmann, S. (2014). Epigenetics: development, dynamics and disease. *Cell Tissue Res*, *356*(3), 451-455. https://doi.org/10.1007/s00441-014-1916-7

Waddington, C. H. (2011). The epigenotype. *International Journal of Epidemiology*, *41*(1), 10-13. https://doi.org/10.1093/ije/dyr184

Waddington, C. H. (2012). The epigenotype. 1942. *Int J Epidemiol*, *41*(1), 10-13. https://doi.org/10.1093/ije/dyr184

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, *181*(2), 281-292.e286. https://doi.org/10.1016/j.cell.2020.02.058

Wang, J., Cui, J., Chen, R., Deng, Y., Liao, X., Wei, Y., Li, X., Su, M., Yu, J., & Yi, P. (2017). Prenatal exposure to lipopolysaccharide alters renal DNA methyltransferase expression in rat offspring. *PloS one*, *12*(1), e0169206. https://doi.org/10.1371/journal.pone.0169206

Wang, J., Yin, N., Deng, Y., Wei, Y., Huang, Y., Pu, X., Li, L., Zheng, Y., Guo, J., Yu, J., Li, X., & Yi, P. (2016). Ascorbic acid protects against hypertension through downregulation of ACE1

gene expression mediated by histone deacetylation in prenatal inflammation-induced offspring. *Sci Rep*, *6*, 39469. https://doi.org/10.1038/srep39469

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K. Y., Wang, Q., Zhou, H., Yan, J., & Qi, J. (2020). Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell*, *181*(4), 894-904.e899. https://doi.org/10.1016/j.cell.2020.03.045

Wang, X., Dinu, I., Liu, W., & Yasui, Y. (2011). Linear combination test for hierarchical gene set analysis. *Stat Appl Genet Mol Biol*, *10*, Article 13. https://doi.org/10.2202/1544-6115.1641

Warskulat, U., Heller-Stilb, B., Oermann, E., Zilles, K., Haas, H., Lang, F., & Häussinger, D. (2007). Phenotype of the taurine transporter knockout mouse. *Methods in enzymology*, *428*, 439-458.

Waterland, R. A., Travisano, M., & Tahiliani, K. G. (2007). Diet-induced hypermethylation at agouti viable yellow is not inherited transgenerationally through the female. *Faseb j*, *21*(12), 3380-3385. https://doi.org/10.1096/fj.07-8229com

Waters, L. S., Minesinger, B. K., Wiltrout, M. E., D'Souza, S., Woodruff, R. V., & Walker, G. C. (2009). Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiology and Molecular Biology Reviews*, *73*(1), 134-154.

Weber-Stadlbauer, U. (2017). Epigenetic and transgenerational mechanisms in infection-mediated neurodevelopmental disorders. *Transl Psychiatry*, *7*(5), e1113. https://doi.org/10.1038/tp.2017.78

Weiss, S. R., & Navas-Martin, S. (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiol Mol Biol Rev*, *69*(4), 635-664. https://doi.org/10.1128/mmbr.69.4.635-664.2005

Weng, X., Liu, F., Zhang, H., Kan, M., Wang, T., Dong, M., & Liu, Y. (2018). Genome-wide DNA methylation profiling in infants born to gestational diabetes mellitus. *Diabetes Research and Clinical Practice*, *142*, 10-18.

West, N. A., Kechris, K., & Dabelea, D. (2013). Exposure to maternal diabetes in utero and DNA methylation patterns in the offspring. *Immunometabolism*, *1*, 1-9. https://doi.org/10.2478/immun-2013-0001

World Health Organization. (2020). Naming the coronavirus disease (COVID-19) and the virus that causes it. *Brazilian Journal of Implantology and Health Sciences*, *2*(3).

World Health Organization, 2. (2020). Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV).

WHO.(2022). Statement on the twelfth meeting of the International Health Regulations (2005) Emergency Committee regarding the coronavirus disease (COVID-19) pandemic.

WHO. (2023). Director-General's opening remarks at the media briefing – 5 May 2023. Retrieved 15 February 2024 from https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing---5-may-2023

WHO.(2024). COVID-19 dashboard. Retrieved 15 February 2024 from https://data.who.int/dashboards/covid19/deaths?n=c

Widagdo, W., Okba, N. M. A., Stalin Raj, V., & Haagmans, B. L. (2017). MERS-coronavirus: From discovery to intervention. *One Health*, *3*, 11-16. https://doi.org/10.1016/j.onehlt.2016.12.001

Wiemels, J. L., Alexander, F. E., Cazzaniga, G., Biondi, A., Mayer, S. P., & Greaves, M. (2000). Microclustering of TEL-AML1 translocation breakpoints in childhood acute lymphoblastic

leukemia. *Genes Chromosomes Cancer*, *29*(3), 219-228. https://doi.org/10.1002/1098-2264(2000)9999:9999<::aid-gcc1028>3.0.co;2-d

Williams, G. M., & Jeffrey, A. M. (2000). Oxidative DNA damage: endogenous and chemically induced. *Regulatory Toxicology and Pharmacology*, *32*(3), 283-292.

Wójcik, O. P., Koenig, K. L., Zeleniuch-Jacquotte, A., Costa, M., & Chen, Y. (2010). The potential protective effects of taurine on coronary heart disease. *Atherosclerosis*, *208*(1), 19-25. https://doi.org/10.1016/j.atherosclerosis.2009.06.002

Wong, S. F., Chow, K. M., Leung, T. N., Ng, W. F., Ng, T. K., Shek, C. C., Ng, P. C., Lam, P. W., Ho, L. C., & To, W. W. (2004). Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. *American journal of obstetrics and gynecology*, *191*(1), 292-297.

Woo, H., Dam Ha, S., Lee, S. B., Buratowski, S., & Kim, T. (2017). Modulation of gene expression dynamics by co-transcriptional histone methylations. *Experimental & molecular medicine*, *49*(4), e326-e326.

Woo, P. C., Lau, S. K., Chu, C. M., Chan, K. H., Tsoi, H. W., Huang, Y., Wong, B. H., Poon, R. W., Cai, J. J., Luk, W. K., Poon, L. L., Wong, S. S., Guan, Y., Peiris, J. S., & Yuen, K. Y. (2005). Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol*, *79*(2), 884-895. https://doi.org/10.1128/jvi.79.2.884-895.2005

Woodward, A. (2020). *A pregnant mother infected with the coronavirus gave birth, and her baby tested positive 30 hours later*. Business Insider. Retrieved 17 February 2024 from https://www.businessinsider.com/wuhan-coronavirus-in-infant-born-from-infected-mother-2020-2

Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., Graham, B. S., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, *367*(6483), 1260-1263. https://doi.org/doi:10.1126/science.abb2507

Wu, H. Y., & Brian, D. A. (2010). Subgenomic messenger RNA amplification in coronaviruses. *Proc Natl Acad Sci U S A*, *107*(27), 12257-12262. https://doi.org/10.1073/pnas.1000378107

Wu, J. T., Leung, K., & Leung, G. M. (2020). Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. *Lancet*, *395*(10225), 689-697. https://doi.org/10.1016/s0140-6736(20)30260-9

Wu, Y., Kang, L., Guo, Z., Liu, J., Liu, M., & Liang, W. (2022). Incubation period of COVID-19 caused by unique SARS-CoV-2 strains: a systematic review and meta-analysis. *JAMA Netw Open*, *5*(8), e2228008. https://doi.org/10.1001/jamanetworkopen.2022.28008

Wu, Y., Liu, C., Dong, L., Zhang, C., Chen, Y., Liu, J., Zhang, C., Duan, C., Zhang, H., Mol, B. W., Dennis, C. L., Yin, T., Yang, J., & Huang, H. (2020). Coronavirus disease 2019 among pregnant Chinese women: case series data on the safety of vaginal birth and breastfeeding. *Bjog*, *127*(9), 1109-1115. https://doi.org/10.1111/1471-0528.16276

Xiao, K., Zhai, J., Feng, Y., Zhou, N., Zhang, X., Zou, J.-J., Li, N., Guo, Y., Li, X., & Shen, X. (2020). Isolation and characterization of 2019-nCoV-like coronavirus from Malayan pangolins. *BioRxiv*, 2020.2002. 2017.951335.

Yamori, Y., Taguchi, T., Hamada, A., Kunimasa, K., Mori, H., & Mori, M. (2010). Taurine in health and diseases: consistent evidence from experimental and epidemiological studies. *J Biomed Sci*, *17 Suppl 1*(Suppl 1), S6. https://doi.org/10.1186/1423-0127-17-s1-s6

Yazigi, A., De Pecoulas, A. E., Vauloup-Fellous, C., Grangeot-Keros, L., Ayoubi, J. M., & Picone, O. (2017). Fetal and neonatal abnormalities due to congenital rubella syndrome: a review of literature. *J Matern Fetal Neonatal Med*, *30*(3), 274-278. https://doi.org/10.3109/14767058.2016.1169526

Yoo, J. Y., Lee, S., Lee, H. A., Park, H., Park, Y. J., Ha, E. H., & Kim, Y. J. (2014). Can proopiomelanocortin methylation be used as an early predictor of metabolic syndrome? *Diabetes care*, *37*(3), 734-739. https://doi.org/10.2337/dc13-1012

Yu, I. T., Li, Y., Wong, T. W., Tam, W., Chan, A. T., Lee, J. H., Leung, D. Y., & Ho, T. (2004). Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N Engl J Med*, *350*(17), 1731-1739. https://doi.org/10.1056/NEJMoa032867

Zeng, H., Xu, C., Fan, J., Tang, Y., Deng, Q., Zhang, W., & Long, X. (2020). Antibodies in infants born to mothers with COVID-19 pneumonia. *JAMA*, *323*(18), 1848-1849. https://doi.org/10.1001/jama.2020.4861

Zentner, G. E., & Henikoff, S. (2013). Regulation of nucleosome dynamics by histone modifications. *Nat Struct Mol Biol*, *20*(3), 259-266. https://doi.org/10.1038/nsmb.2470

Zhang, Y., Guo, R., Kim, S. H., Shah, H., Zhang, S., Liang, J. H., Fang, Y., Gentili, M., Leary, C. N. O., & Elledge, S. J. (2021). SARS-CoV-2 hijacks folate and one-carbon metabolism for viral replication. *Nature communications*, *12*(1), 1676.

Zhang, Y. Z., & Holmes, E. C. (2020). A genomic perspective on the origin and emergence of SARS-CoV-2. *Cell*, *181*(2), 223-227. https://doi.org/10.1016/j.cell.2020.03.035

Zhao, Y., & Garcia, B. A. (2015). Comprehensive catalog of currently documented histone modifications. *Cold Spring Harbor perspectives in biology*, *7*(9), a025064.

Zhou, S., Lu, H., & Xiong, M. (2021). Identifying immune cell infiltration and effective diagnostic biomarkers in rheumatoid arthritis by bioinformatics analysis. *Front Immunol*, *12*, 726747. https://doi.org/10.3389/fimmu.2021.726747

Zhou, T., Tsybovsky, Y., Gorman, J., Rapp, M., Cerutti, G., Chuang, G. Y., Katsamba, P. S., Sampson, J. M., Schön, A., Bimela, J., Boyington, J. C., Nazzari, A., Olia, A. S., Shi, W., Sastry, M., Stephens, T., Stuckey, J., Teng, I. T., Wang, P., . . . Kwong, P. D. (2020). Cryo-EM structures of SARS-CoV-2 spike without and with ACE2 seveal a pH-dependent switch to mediate endosomal positioning of receptor-binding domains. *Cell Host Microbe*, *28*(6), 867-879.e865. https://doi.org/10.1016/j.chom.2020.11.004

Zhu, Z., & Cao, F. (2019). Epigenetic programming and fetal metabolic programming. *Frontiers in endocrinology*, *10*, 422860.

Zijlstra, G. J., Hacken, N. H. T. t., Hoffmann, R. F., Oosterhout, A. J. M. v., & Heijink, I. H. (2012). Interleukin-17A induces glucocorticoid insensitivity in human bronchial epithelial cells. *European Respiratory Journal*, *39*(2), 439-445. https://doi.org/10.1183/09031936.00017911

Zilbauer, M. (2014). Epigenetics--a novel concept with exciting prospects for paediatric research. *Arch Dis Child Educ Pract Ed*, *99*(2), 67-69. https://doi.org/10.1136/archdischild-2013-304539

Zilbauer, M., Zellos, A., Heuschkel, R., Gasparetto, M., Kraiczy, J., Postberg, J., Greco, L., Auricchio, R., Galatola, M., Embleton, N., Wirth, S., & Jenke, A. (2016). Epigenetics in Paediatric Gastroenterology, Hepatology, and Nutrition: Present Trends and Future

Perspectives. *J Pediatr Gastroenterol Nutr*, *62*(4), 521-529. https://doi.org/10.1097/mpg.0000000000001053

Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., ... & Wu, J. (2020). SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *New England journal of medicine*, 382(12), 1177-1179.