European Journal of Obstetrics & Gynecology and Reproductive Biology: X 8 (2020) 100118

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology: X

journal homepage: www.elsevier.com/locate/eurox

Blood gene transcript signature profiling in pregnancies resulting in preterm birth: A systematic review

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ARTICLE INFO

Article history:
Received 27 May 2020
Received in revised form 16 September 2020
Accepted 21 September 2020
Available online 22 September 2020

Keywords:
Antenatal screening
Gene expression profiling
Preterm birth
Systematic review
Transcriptome

ABSTRACT

Objective: To pursue a systematic review and summarise the current evidence for the potential of transcriptome molecular profiling in investigating the preterm phenotype.
Study design: We systematically reviewed the literature, using readily available electronic databases (i.e. PubMed/Medline, Embase, Scopus and Web of Science) from inception until March 2020 to identify investigations of maternal blood-derived RNA profiling in preterm birth (PTB). Studies were included if circulating coding or non-coding RNA was analysed in maternal blood during pregnancy and/or at delivery. Interventional trials were not included. The primary outcome was the availability of whole genome expression patterns evaluated in pregnancies resulting in preterm deliveries.
Results: A total of 35 articles were included in the final analysis. Most of the studies were conducted in high-income countries and published in the last decade. Apart from spontaneous PTB, a variety of phenotypes leading to preterm delivery were reported. Differences in sampling methods, target gene selection and laboratory protocols severely limited any quantitative comparisons. Most of the studies revealed that gene expression profiling during pregnancy has high potential for identifying women at risk of spontaneous and/or non-spontaneous PTB as early as in the first trimester.
Conclusion: Assessing maternal blood-derived transcriptional signatures for PTB risk in pregnant women holds promise as a screening approach. However, longitudinally followed, prospective pregnancy cohorts are lacking. These are relevant for identifying causes leading to PTB and whether prediction of spontaneous PTB or co-morbidities associated with PTB is achievable. More emphasis on widely employed standardised protocols is required to ensure comparability of results.

Contents

Abbreviations: ANC, antenatal care; DNA, deoxyribonucleic acid; EGA, estimated gestational age; FGR, fetal growth restriction; HIC, high-income country; LIC, low-income country; LMP, last menstrual period; MIC, middle-income country; miRNA, microRNA; mRNA, messenger RNA; NGS, next generation sequencing; PCR, polymerase chain reaction; PICo, Population phenomenon of Interest and Context; PPROM, preterm premature rupture of membranes; PROSPERO, Prospective Register of Systematic Reviews; PTB, preterm birth; PTL, preterm labour; PoA, proportion of agreement; RIN, RNA integrity number; RNA, Ribonucleic acid; SDG, Sustainable Development Goal; SGA, small for gestational age; sPTB, spontaneous preterm birth; sPTL, spontaneous preterm labour; WBC, white blood cells; WHO, World Health Organization.

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http://dx.doi.org/10.1016/j.eurox.2020.100118

1. Introduction

The World Health Organization (WHO) defines preterm birth (PTB) as birth before completion of 37 weeks of gestation or fewer than 259 days since the first day of the last menstrual period (LMP) [1]. PTB is the leading cause of new-born mortality and morbidity, and it remains the single most important risk factor for death in children under the age of 5 years [2,3]. Even late PTB, from 32 but before 37 weeks' gestation is associated with an increased risk of morbidity beyond the neonatal period, higher rates of re-hospitalisation, and with subsequent socioeconomic ramifications [1,4–6].

Globally, PTB affects approximately 10 % of all pregnancies [7–9], which translates to an estimated number of 14.84 million (uncertainty interval 12.65 million–16.73 million) in 2014 [9]. PTB is nested into Sustainable Development Goal (SDG) 3, which calls for an end to avertable child and neonatal deaths by 2030 [10].

Interestingly, no clear pattern of PTB incidence can be derived from comparing high-income-countries (HICs) with low income-countries (LICs), and a reporting bias due to divergent PTB definitions must be considered. In the absence of advanced neonatal care, the mortality rate for extreme preterm remains near 100 % in LICs [11] and PTBs at home, may never be registered [8]. Limited access to research in LICs prevents benefit from advanced clinical translational research [12].

Optimal antenatal care (ANC) is a prerequisite to alleviate pregnancy-associated complications, including reduction of the PTB risk [13]. So far predictive clinical risk models are insufficient to identify PTB risk [14]. Other approaches include assessment of vaginal microbiome, the state of the maternal immune system via mass cytometry and protein biomarkers derived from maternal blood or blood components [15–18]. While some of these methods are promising, wide scale implementation has not been achieved.

In recent years, gene expression profiling, which allows in-depth phenotyping, has taken an important role as a potential tool to predict health outcomes [19]. The transcriptome is the aggregate of the entire RNA transcribed from the DNA. This transcription and the subsequent translation into effector proteins, is a perpetual process that is influenced by physiological changes (e.g. pregnancy), communicable as well as non-communicable diseases and environmental exposures. Transcriptome profiling enables inferences on the status of various tissues; however, a limitation is lacking anatomical accessibility to the tissues of interest (e.g. myometrial or placental cells). In contrast, blood transcriptome samples can be easily obtained at any time.

Improvement of laboratory protocols enable assessment of whole blood transcriptome profiles in very small amounts of blood [20,21]. RNA transcribing blood leukocytes relate information on the status of the immune system [22], a key player in embryo implantation and placentation, promotion of fetal growth and initiation of labour and delivery [23]. Furthermore, circulating placental RNA and fetal genetic material is increasingly used in prenatal screening procedures [24,25]. The points listed above provide an avenue for in-depth gene expression profiling in pregnancy. Current knowledge gaps include (i) understanding the causes and risk factors for PTB; (ii) assessing transcriptome profiling as a predictive tool for PTB; and (iii) informing on timing of blood transcriptome assessment during pregnancy to predict and guide targeted interventions for PTB. We summarise the body of evidence by systematically evaluating published literature in which the preterm phenotype was investigated by gene expression in maternal blood or immune cell populations isolated from maternal blood.

2. Methods

A protocol outlining the rationale, objectives and search strategies of this systematic review was deposited in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42019122962 [26].

2.1. Search strategy and selection process

The search strategy followed a **P**opulation, phenomenon of **I**nterest and **Co**ntext (PICo) approach to identify relevant published research [27]. For this systematic review, we searched PubMed/MedLine, Embase, Scopus and Web of Science databases from inception to March 2020.

The following terms and Boolean operators were used to execute the search: pregnan* OR gravid* AND preterm OR premature AND RNA OR mRNA OR "cell free nucleic acid*" OR "circulating nucleic acid*" OR "gene expression" OR transcriptom* OR microarray OR "RNA seq" OR RNAseq OR "RNA sequencing" AND blood OR leucocyte* OR PBMC OR neutrophil* OR monocyte* OR lymphocyte* OR T-cell* OR B-cell* OR NK-cell* OR "dendritic cell*".

The PICo table, search strategy and numbers retrieved are available in the Appendix. The search was conducted by the first author (TB) under the supervision of the second last author (RM). After the search was executed, an E-mail alert was set up to identify potential new records, while the review was ongoing. A bibliographic management software (Zotero, version 5.0.54) was used to collate and maintain extracted literature.

Studies that measured genome/genome-wide, circulating coding and/or non-coding RNA in human maternal blood samples

anytime during pregnancy and delivery, and evaluated preterm as a pregnancy outcome were included. Observational, cross-sectional, case-control or cohort design were considered, while interventional trials were excluded. In addition, included studies had to be published in peer-reviewed literature as original articles with the full text available in English language. No temporal or geographic restrictions were applied.

Titles and abstracts were screened, and manuscripts excluded if the content did not conform to the aforementioned criteria. For final inclusion, full texts were evaluated by two independent reviewers (TB and BSAK), who were blinded to each other. Discrepancies were resolved with a third reviewer (RM). If the full text of a study was not available, the authors were contacted to request a copy of the full text.

2.2. Subgroup allocation

To address the specific objectives and aims of included studies, a subgroup allocation was organised. Each article was assessed for whether maternal blood transcriptomics (i) contributed to identifying factors leading to PTB (group 1); (ii) allowed prediction of PTB or preterm labour (PTL) (group 2); and (iii) at what gestational age a transcriptome screening was recommended (group 3). Allocation to several groups was allowed.

2.3. Data collection

After study selection, two independent reviewers (TB and BSAK) extracted data in duplicate by using a predefined data extraction form (Table A8).

2.4. Definitions

PTB was defined as pregnancy outcome before completion of 37 weeks of gestation. Economic country profiles were based on the definitions of the World Bank list of economies (June 2018).

2.5. Risk of bias assessment

As no randomised trials were included in this systematic review, traditional systematic biases (e.g. selection bias, allocation concealment bias, etc.) were not assessed for the selected papers. Risk of bias for included observational, case-control or nested case-control studies was assessed by a modified Newcastle-Ottawa scale (Fig. A1) [28].

2.6. Statistical analysis

Data are presented as absolute number and proportion where applicable. Coefficients of interrater agreement were determined (R package *irr*, version 0.84.1, [29]) and expressed by crude proportion of agreement (PoA) and kappa (κ) statistics (Table A5).

3. Results

Databases were searched in January 2019 and new records identified until March 2020. After the original search and removal of duplicates, 1743 records were identified for screening, of which 1698 items were excluded via title and abstract screening. Overall, full texts of 49 articles were assessed, of which 35 were eligible for inclusion (Fig. 1 and Table A1-A4).

3.1. Study characteristics

Identified studies were published between 2005 and 2019 and most originated from HICs (82.9 %, 29/35), with the remainder from middle-income countries (MICs; 17.1 %, 6/35) (Table 1). No studies from LICs were identified.

Two papers reported observational approaches, while the remaining studies followed a case-control or a nested case-control study design. Studies that assessed gene expression in multiple tissues, extracted and reported samples sizes considered only subgroups in which maternal whole blood or isolated cell subpopulations were analysed. A wide range of sample sizes was reported, with the minimum being 18 as reported by Truong and colleagues [30] and the largest published by Pandey *et al.* [31] at a size of 1118 with 559 cases and 559 controls.

A wide scope of demographic and clinical data were reported and various phenotypes leading to PTB were investigated. Among these phenotypes were (i) spontaneous PTB (sPTB) or spontaneous PTL (sPTL); (ii) PTB with labour; (iii) PTB without labour; (iv) PTB with premature rupture of membranes (PPROM); (v) PTB due to preeclampsia; (vi) PTB due to fetal growth restriction (FGR); (vii) PTB associated with infections (e.g. chorioamnionitis); (viii) PTL with subsequent term delivery; and (ix) iatrogenic PTB due to other medical indications. The column showing sample sizes in Table 1 indicates analysed subgroups and number included in the respective subgroup. In 30 out of 35 studies (85.7 %), blood samples were taken at a single time point. One study reported two [32] and another one reported four [33] sampling timepoints, respectively. Serial sampling was reported by two studies [34,35] while in the project reported by Pacheco and colleagues, the number of sampling timepoints was dependent on time elapsed between hospital admission and delivery [36].

In 71.4 % (25/35), multiple pregnancies were excluded, while in 28.6 % (10/35) this was not specified. Accurate estimation of the gestational age is pivotal in the assessment of PTB; however, the method of estimated gestational age (EGA) confirmation was not mentioned in 62.9 % (22/35) of studies. Otherwise, gestational age was estimated by LMP, ultrasound confirmation or a combination of both. In some instances, the authors referred to procedures as recommended by specialist medical associations (e.g. The American College of Obstetricians and Gynecologists) or, in the seven nested case-control studies, three referenced procedures in the parent studies.

Reporting of central tendency for EGA at delivery was not standardised. On occasion, ranges of EGA of preterm deliveries were not reported at all (8.6 %, 3/35), units were not added to reported numbers (8.6 %, 3/35), only definitions, but no actual results were reported (11.4 %, 4/35) or it was not clear whether the reported central tendency reflected EGA at birth or at the sampling timepoint (2.9 %, 1/35).

Winger et al. [37] included pregnancy outcomes of <38 weeks EGA in the preterm group. However, since a subgroup of early PTB (<34 weeks EGA) was reported and compared to other groups, it was decided to include this paper in the review.

3.2. Technical and methodical aspects

Methodological aspects were more detailed than demographic and clinical parameters (Table A7). Array based technologies (e.g. microarray) were used in nine instances, nucleic acid amplification-based methods (e.g. quantitative PCR) in 24 and RNAseq in six papers. On one occasion the nCounter® platform (nanoString Technologies; Seattle, USA) was used [38]. In six studies more than one technology was used (e.g. separate platforms for target identification and verification).

On 19 occasions researchers took whole blood from expectant mothers, plasma in eight, isolated white blood cells (WBC) in five, serum in one and a combination of whole blood and isolated monocytes in two studies. A range of 2–20 ml of blood was taken and in 22.9 % (8/35) of the articles the total amount of blood was

not stated. In six studies, the amount was retrieved from the manufacturer of sampling tubes used.

Gene expression in multiple tissues was assessed in 31.4 % (11/35) of the studies. Bukowski et al. included a total of seven different tissues in their analysis (i.e. whole blood, placenta, chorion, decidua, amnion, fetal blood and myometrium) [39].

PAXgene, a system for immediate stabilization of intracellular RNA, was the most commonly reported tool for RNA preservation (42.9 %, 15/35), followed by TRIzol LS in 8.6 % (3/35), RNAlater (Thermo Fisher Scientific; Waltham, USA) and TRI Reagent BD (Sigma-Aldrich; St. Louis, USA) in 2.9 % (1/35). In 42.9 % (15/35) of the studies included, no RNA preservation system was used or reported.

Different RNA extraction protocols and platforms for RNA quantification were used and a variety of coding and non-coding RNA transcripts were targeted. In 60.0 % (21/35) of publications RNA quantity was measured, but results were reported in only one instance [40]. Similarly, RNA quality was determined in 31 % (11/35) of studies, but only one study [40] provided an RNA integrity number (RIN) and another reported that RNA quality was above a certain threshold [32]. Objectives and research questions determined the selection of RNA targets, and the number of selected RNA targets ranged from one target gene (20.0 %, 7/35), to thousands of targets printed on array-based platforms or quantification by RNA sequencing.

3.3. Subgroup allocation

To investigate the nature of gene expression profiling, the included papers were divided in three subgroups.

3.3.1. Group 1 – improvement of understanding of causes leading to PTB

A wide range of methodical designs with different RNA targets and phenotypes describe their pathways leading to PTB. Consequently, the molecular pathways characterised by over- or under-expressed genes may reflect the underlying condition rather than the direct causes leading to sPTB. To summarise the effector functions of significantly altered genes and their associated biological pathways, keywords of reported pathways and gene functions were extracted and compiled (Fig. 2). Corroborating data on PTB and labour in general, the most commonly identified tag words revolved around the immune system, its biological function and *inflammation*. A summary of major findings is provided in Table 2.

3.3.2. Group 2 – predictability of PTB by assessing blood derived gene expression

Apart from one study that aimed at identifying reference genes for normalizing circulating RNA levels in maternal blood [41], all

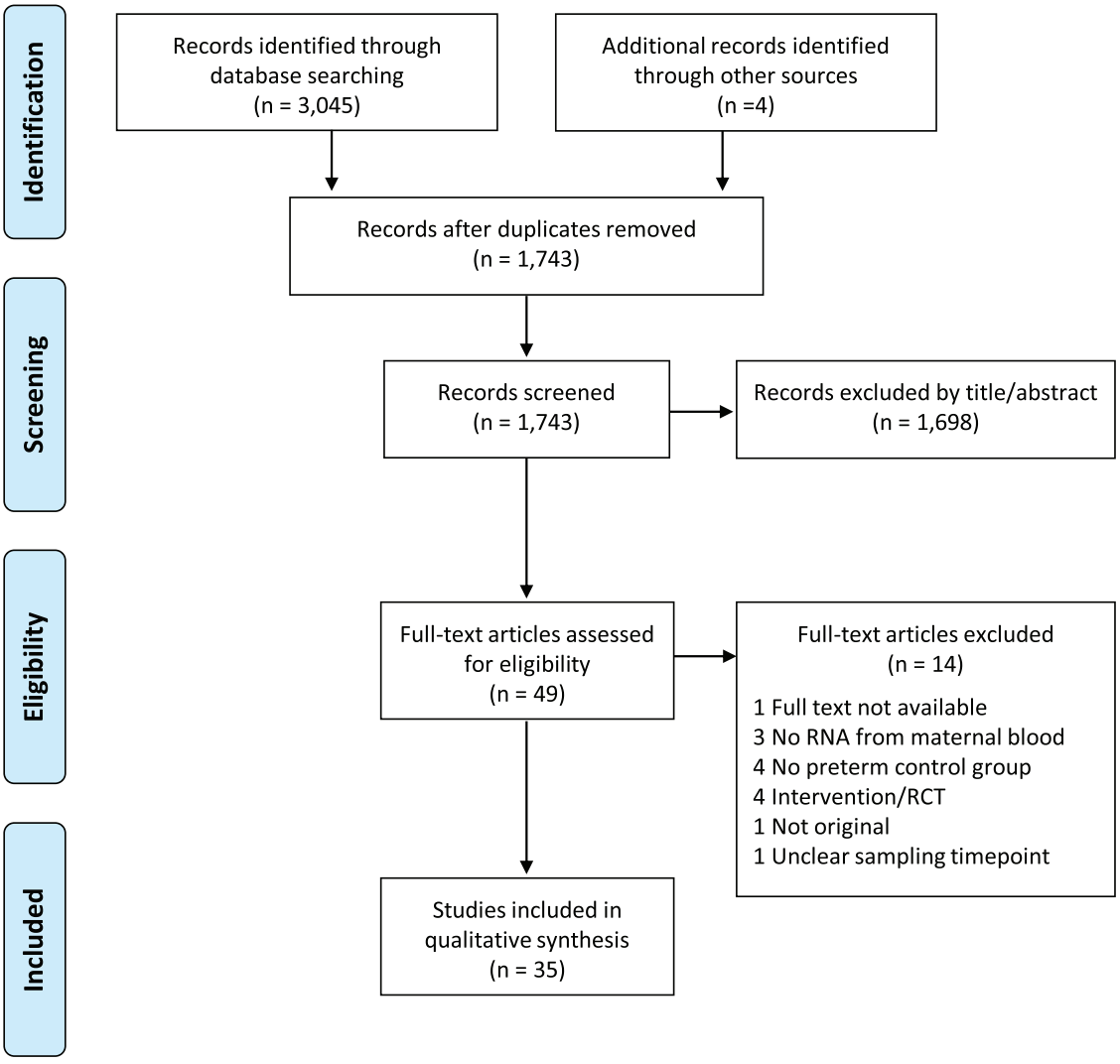

Fig. 1. Study selection flow diagram according to PRISMA 2009 (Moher 2009).
Databases were searched in January 2019 and new records were identified via an automated alert system until March 2020.

Table 1
Characteristics of included studies.

Reference	Country	ECP	Type of study	Sample size	Average EGA in weeks	No sampling tp	NoF	EGA confirmation
Bukowski 2017	USA	HIC	Case-control	35 Total 8 sPTB with labour 10 PTB without labour (elective) 7 term with labour 10 term without labour	Definitions reported but results not reported	1	1	NR
Chim 2012	Hong Kong SAR, China	HIC	Case-control	37 Total 10 sPTB 27 term	30.9 (27.0–32.1) Median (IQR) Plasma sample group	1	1	LMP, US
Chim 2017	South Korea	HIC	Observation	20 sPTL	NR	1	1	LMP, US
Dahlstrom 2010	Norway	HIC	Case-Control	24 Total 8 PTB CS (preeclampsia) 8 elective CS at term 8 term birth at EGA 29	32 (29–36) Mean (NR)	1	NR	NR
Elovitz 2015	USA	HIC	Nested case-control	80 Total 40 sPTB 40 term	27.3 (25.75–29.35) Median (IQR)	1	1	NR
Enquobahrie 2009	USA	HIC	Nested case-control (Pilot)	30 Total 4 sPTB 5 PTB with PROM 5 other PTB 16 term	32.3 (2.1) Mean (NR)	1	1	LMP, US
Gratton 2016	Australia	HIC	Case-control (for blood mRNA)	55 Total 30 PTB with preeclampsia 25 EGA matched term	EGA not reported for cohort with blood sampling	1	NR	NR
Gray 2017	New Zealand	HIC	Nested case-control	24 Total 7 sPTB included in analysis 9 term	31.4 (0.6) Mean (SEM)	1	1	US¶
Heng 2014	Australia	HIC	Case-control	154 Total 48 sPTB within 48 h of admission 12 sPTB>2, <7 days of admission 15 sPTB > days, < 37 weeks EGA 79 term	 31.8 ± 3.3 29.5 ± 3.1 34.8 ± 2.1 Mean ± SD	1	1	NR
Heng 2016	Canada	HIC	Nested case-control	165 Total 15 sPTB 36 PPROM 114 term	33.6 ± 2.6 Mean ± SD	2	1	NR
Menon 2019	India	LMIC	Nested case-control	30 Total 10 sPTB 20 term	36.0 (35.0–36.3) Median (IQR)	4	1	US
Mustafa 2015	India	LMIC	Case-control	100 Total 50 sPTB 50 term	NR	1	1	NR
Ngo 2018	Denmark USA	HIC	Observation	38 Total 13 sPTB 25 term	Two sites: - 26.4 ± 2.3 - 30.6 ± 2.4 Mean ± SD	1 (PTL set) weekly (EGA prediction set)	1	LMP, US*
Nowicki 2009	USA	HIC	Case-control	75 Total 34 sPTL 11 PTL with infection 30 controls without PTL	NR	1	NR	NR

Table 1 (*Continued*)

Reference	Country	ECP	Type of study	Sample size	Average EGA in weeks	No sampling tp	NoF	EGA confirmation
Pacheco 2011	USA	HIC	Case-control	60 Total 30 sPTL 30 term	Definitions reported but results not reported	1–3 (depending on time of delivery after admission)	1	LMP, US
Paiva 2011	Australia	HIC	Case-control	64 Total 15 preterm preeclampsia 15 EGA matched term 8 EGA 13–15 (low risk pregnancy) 17 EGA 28 (low risk pregnancy) 9 term	31.1 (3.9) Mean (SEM)	1	1	NR
Pandey 2017	India	LMIC	Case-control	1118 Total 559 sPTB 559 term	33.96 ± 1.78 Units not reported	1	1	NR
Paquette 2018	Canada	HIC	Nested case-control	50 Total 20 sPTL 30 term	Definitions reported but results not reported	1	1	US[¶]
Paquette 2019	Canada	HIC	Nested case-control	45 Total 15 sPTL 30 term	28.78 ± 2.97 Mean ± SD	1	1	US[¶]
Pawelczyk 2010	USA	HIC	Case-control	102 Total 41 sPTL 41 EGA matched term 8 term in labour 12 term not in labour	Time of sampling 31.2 (10) Median (IQR)	1	NR	NR
PrearoMoco 2018	Brazil	UMIC	Case-control (cross-sectional in methods)	40 Total 20 sPTL with PTB 20 term (EGA matched)	Time of sampling 34.1 (25.6–36.6) Median (Range)	1	1	LMP, US
Stock 2015	Australia	HIC	Case-control	39 Total 19 PPROM (chorioamnionitis) 8 PPROM (no chorioamnionitis) 12 term (EGA matched)	 28.6 ± 3.7 32.0 ± 2.8 Mean ± SD	Serial (recruitment to delivery)	NR	NR
Tiwari 2016	India	LMIC	Case-control	209 Total 14 extremely PTB 36 very PTB 59 moderately/late PTB 100 term	Definitions reported but results not reported	1	NR	NR
Truong 2017	USA	HIC	Case-control	18 Total 6 sPTB 6 preeclampsia term 6 term	35 ± 1.1 Mean ± SD	1	NR	NR
Tsai 2017	Taiwan, China	HIC	Case-control	139 Total 29 sPTB 31 preeclampsia term and PTB 19 SGA term and PTB 60 term	34.73 ± 2.00 Mean ± SD	1	NR	NR
Tyagi 2016	India	LMIC	Case-control	60 Total 30 sPTB 30 term	34.8 ± 1.68 Mean ± SD	1	NR	NR
Whitehead 2013a	Australia	HIC	Case-control	50 Total 8 PTB FGR with preeclampsia	29.5 (21) Mean (SD)	1	NR	NR

				12 PTB FGR without preeclampsia 30 term (EGA matched)				
Whitehead 2013b	Australia	HIC	Case-control	24 Total 12 PTB FGR 12 term (EGA matched)	30.1 (3) Mean (SEM)	1	1	NR
Whitehead 2013c	Australia	HIC	Case-control	40 Total 20 FGR PTB 20 term (EGA matched)	29.5 (3) Mean (SEM)	1	1	US
Whitehead 2013d	Australia	HIC	Case-control	40 Total 20 PTB FGR 20 Term controls	29. 5 (3) Mean (SEM)	1	1	NR
Whitehead 2013e	Australia	HIC	Case-control	43 Total 20 PTB FGR 15 term (EGA matched) 8 term (at delivery)	29.5 (3) Mean (SEM)	1	1	NR
Winger 2017	USA	HIC	Case-control	39 Total 14 sPTB 29 term	33.6 ± 2.9 Mean ± SD	1	1	NR
Wommack 2018	USA	HIC	Case-control	42 Total 21 sPTB 21 term	35.3 ± 2.4 Mean ± SD	1	1	NR
Yuan 2009	United Kingdom	HIC	Case-control	37 Total 7 sPTB in labour 10 sPTB not in labour 10 term not in labour 10 term in labour	Unclear if EGA at sampling or delivery	1	1	US
Zhong 2005	Switzerland	HIC	Case-control	50 Total 11 PTB 24 PTL, term delivery 15 term	33 Median	1	1	US

If multiple tissues were analysed, results for are reported only for blood samples. If multiple phenotypes were analysed, total sample size and samples sizes of subgroups are highlighted with bullet points. Average EGA is presented in weeks and in the event of different average EGA for subgroups, the average EGA is presented next to the respective subgroup.

* According to American College of Obstetricians and Gynecologists guidelines.

¶ From the parent cohort study.

Abbreviations: ACOG, American College of Obstetricians and Gynecologists; CS, caesarean section; ECP, economic country profile; EGA, estimated gestational age; FGR, fetal growth restriction; NoF, number of fetuses; HIC, high-income country; h, hours; IQR, interquartile range; LMIC, low- and -middle-income country; LMP, last menstrual period; mRNA, messenger RNA; No sampling tp, number of sampling timepoints; NR, not reported; PPROM, preterm premature rupture of membranes; PROM, premature rupture of membranes; PTB, preterm birth; PTL, preterm labour; SAR, special administrative region; SD, standard deviation; SEM, standard error of the mean; SGA, small for gestational age; sPTB, spontaneous preterm birth; UMIC, upper-middle income country; US, ultrasound.

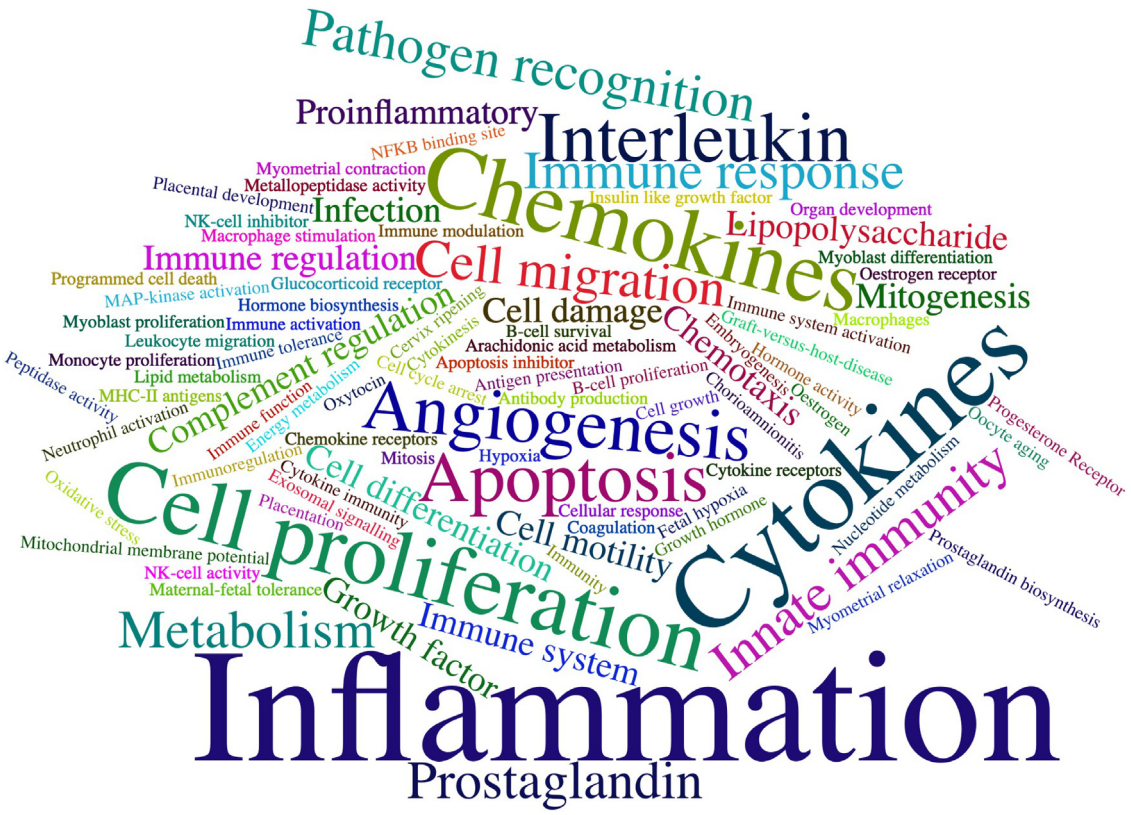

Fig. 2. Keywords of most commonly encountered gene functions and associated biological pathways. Font size is proportional to the number of times the words were encountered.

papers referred to whether assessing maternal blood transcriptome profiles in pregnant woman supports prediction of PTB. Elovitz *et al.* assessed microRNA profiles in sPTB and concluded that profiling of miRNAs is unlikely to become a useful biomarker for prediction of PTB [42], all other papers indicated that prediction is potentially possible. Spontaneous PTB was the primary outcome in 57.1 % (20/35) of studies, and an inference on the predictability of sPTB was possible. Spontaneous PTB was one of multiple groups assessed in three studies, while in 10 an underlying morbidity served as a surrogate for PTB (i.e. preeclampsia, hypoxia and FGR, SGA, inflammation based on infection or increased organophosphate exposure). If study results permitted a prediction, the proportion of cases identified correctly by RNA expression profiling ranged from 60 % to 100 %, the latter reported by Winger et al. for early sPTB at <34 weeks of EGA [37].

3.3.3. Group 3 – timing of sampling

Most of the studies performed single timepoint sampling. Repeated sampling was done in 14.3 % (5/35) of the studies. Two studies initiated sampling after symptoms of PTL or threatening PTB were present [34,36]. Serial sampling was done in a sub-cohort that did not experience a PTB [35]. Multiple sampling timepoints at predefined timepoints was reported only in two papers [32,33].

4. Discussion

This systematic review complied results from research investigating the PTB phenotype by means of gene expression profiling in samples derived from maternal blood. While restriction to English language is a limitation to presenting all the evidence on this subject globally, it is unlikely to have resulted in a systematic bias. A quantification of results of extracted papers in the form of a meta-analysis was not achievable due to the substantial methodical differences and various investigated phenotypes leading to PTB. Hence, the interpretation of this narrative review was restricted to observational description of the extracted evidence.

It is notable that no study from a LIC was identified considering that some LICs report the highest PTB rates and lowest survival of these infants [9,43]. We conjecture that LICs would disproportionally benefit from identification of expectant mothers at an increased risk of PTB and targeted interventions guided by improved understanding of factors leading to PTB. Due to genomic variation, gene expression is not generalisable [44]; hence, populations in LICs should be granted equal access to research in order to confirm research from other populations or to identify population based differences.

Spontaneous PTB cases are summarised as cases of sPTL and PPROM, with no induction of labour [45]. In 57.1 % (20/35) of the studies included, sPTB was the only phenotype assessed. Otherwise, primary objectives included various phenotypes aside from sPTB, or multiple phenotypes as well as control groups. The disparity in case selection prohibits a direct comparison of these studies. As reported comorbidities (e.g. preeclampsia, FGR, SGA, fetal hypoxia, etc.) lead to induced preterm birth, they were included in this narrative review. Of the 15 reports that analysed gene expression in different PTB subgroups, reported biological pathways involved and predictability were similar. Early identification of specific aetiologies for PTB would enable early management of non-sPTB.

Non-uniformity of laboratory methods also prevents a quantitative interpretation. This includes the selection of different coding and non-coding RNAs, but also to the various sample preparations and RNA preservations used.

The overall diversity of the studies was reflected by the wide range of sample sizes. The trade-off between depth and breadth in

Table 2
Summary of primary objectives and major findings.

Reference	Primary objective	Major finding	Group	Sensitivity estimate
Bukowski 2017	to " . . . comprehensively evaluate the mRNA transcriptome that characterizes preterm and term labour in tissues comprising the pregnancy using precisely phenotyped samples."	" . . . gene expression differences among the four phenotypes were highest in the decidua, amnion and chorion rather than in the fundus and lower segment of the uterus or in the maternal or fetal blood." AND " . . . pregnancy is maintained by downregulation of chemokines at the maternal-fetal interface."	1, 2	NR for blood
Chim 2012	1.″To decipher if certain genes were associated in (a) the pathogenesis of SPB, and/or (b) the normal term spontaneous labor process . . . "2.″ To investigate if the SPB-associated RNA that could be detected before SPB, . . . "	" . . . SPB- associated RNA in maternal plasma could be detected before SPB eventually occurred . . . "	1, 2	60 % (6/10)
Chim 2017	" . . . a search for reference genes suitable for the normalization of RT-qPCR data on whole blood collected from women during their presentation of preterm labor"	1.″ . . . a panel of 395 genes, . . . , were identified to comprise exons with considerably less variable expression level . . . than any GAPDH exon" 2.″This panel is over-represented with genes involved with the actin cytoskeleton, macromolecular complex, and integrin signaling."	NA	NA
Dahlstrom 2010	" . . . to evaluate genome signaling in blood during preeclampsia and towards term using microarrays."	1.″ . . . women with early onset preeclampsia and women with normal pregnancies towards term both have distinct genome expression patterns in blood when compared to normal pregnancy at gestational week 31.″ 2.″A possible type 1 immune response was identified both during preeclampsia and towards term."	1, 2	NR
Elovitz 2015	" . . . to determine whether miRNA profiles in maternal blood are different in women who are destined to have a preterm, compared with a term, birth"	" . . . demonstrated that miRNA profiles in maternal serum are not significantly different in women who are destined to have a preterm delivery compared with a term birth"	1, 2	NR
Enquobahrie 2009	" . . . evaluated transcriptional gene expression patterns associated with PTD . . . to develop predictive tools for PTD. Functions and functional relationships of differentially expressed genes were investigated to better understand pathophysiologic processes underlying PTD."	"PTD is associated with maternal early pregnancy peripheral blood gene expression changes. . . . blood gene expression patterns may be useful for better understanding of PTD pathophysiology and PTD risk prediction."	1, 2	65–69%
Gratton 2016	" . . . explores a role for STS (stroid sulfatase) in preeclampsia. . . . investigated whether STS mRNA is detectable in maternal whole blood"	" . . . STS mRNA expression was significantly increased in preeclamptic whole blood compared to normal healthy controls"	1, 2	NR
Gray 2017	" . . . to explore the potential of circulating miRNAs as biomarkers during early pregnancy to predict those individuals that go on to experience a later SPTB"	" . . . data suggest that unique circulating miRNA profiles may provide attractive candidates as putative biomarkers for prediction of SPTB risk during early pregnancy."	1, 2	NR
Heng 2014	" . . . to study differential whole blood gene expression associated with spontaneous preterm birth (sPTB) within 48 h of hospital admission."	" . . . model to predict sPTB was achieved using the top nine differentially expressed genes coupled with peripheral clinical blood data (sensitivity 70.8 %, specificity 75.5 %). These differentially expressed genes may further elucidate the underlying mechanisms of sPTB and pave the way for future . . . studies to predict sPTB"	1, 2	70.8 % (predicted delivery within 48 h of admission, coupled with clinical blood data)
Heng 2016	"The aim of this study was to investigate maternal whole blood gene expression profiles associated with spontaneous preterm birth (SPTB, <37 weeks) in asymptomatic pregnant women."	" . . . work has shown that clinical factors and whole blood gene expression are associated with SPTB in asymptomatic women. Gene set enrichment analyses revealed elevated inflammation in women who had SPTBs."	1, 2, (3)	64.7 % (comparing fold change between T2 and T3, including clinical factors)
Menon 2019	" . . . to discover exosome miRNA cargoes that are differentially expressed in total maternal plasma to generate a profile of their longitudinal changes during each stage of gestation and real-time insight into functional changes associated with gestational age in	"The data . . . establish that circulating exosomes carry a specific set of miRNAs as a function of the gestational age in term pregnancy, and that the circulating exosomal miRNA profile changes in PTB pregnancies compared with normal term deliveries."	1, 2, (3)	NR

Table 2 (*Continued*)

Reference	Primary objective	Major finding	Group	Sensitivity estimate
	term and preterm birth (PTB) pregnancies."			
Mustafa 2015	" . . . to explore associations of blood concentrations of organochlorine pesticides (OCPs) with inflammatory/ antioxidant gene expression, and cytokines and prostaglandin levels in PTB cases."	"Significantly high levels of . . . "organophosphates " . . . , increased expression of cyclooxygenase-2 (COX-2), and decreased expression of manganese superoxide dismutase (Mn-SOD) and catalase (CAT) genes were seen in PTB cases."	1, 2	NA
Ngo 2018	. . . whether "noninvasively measuring cell-free RNA (cfRNA) transcripts from fetal tissues in maternal blood" . . . "can be developed into blood tests that establish gestational age and estimate the risk of preterm birth"	" . . . measurement of nine cell-free RNA (cfRNA) transcripts in maternal blood predicted gestational age with comparable accuracy to ultrasound". " . . . identified seven cfRNA transcripts that accurately classified women who delivered preterm up to 2 months in advance of labor."	1, 2, (3)	75 % (6/8) first dataset 80 % (4/5) second dataset
Nowicki 2009	" . . . explored the association between PTL/PTB and the "activation" of the peripheral circulatory system by determining whether CD55 mRNA expression within peripheral WBC's differed between PTL and control patients not in labor "	" . . . CD55 mRNA expression was elevated in the peripheral WBC's of subjects with preterm labor . . . and that elevated leukocyte CD55 may be a useful predictor of subsequent PTB."	1, 2	69 % for PTL, 81 % for infection associated PTL, 73 % for PTL resulting in PTB
Pacheco 2011	" . . . to determine the kinetics of DAF expression on maternal WBCs in women with a clinical diagnosis of PTL."	" . . . PTL is associated with a significant increase in expression of DAF in peripheral WBCs."	1, 2	NR
Paiva 2011	" . . . to identify a panel of genes highly expressed in the placenta and compare their expression in placenta and maternal whole blood from PE vs. control pregnancies."	" . . . genes highly expressed in the placenta may be promising candidates as circulating mRNA biomarkers of PE."	1, 2	NR
Pandey 2017	" . . . to assess the association of anti-inflammatory cytokine IL-10 gene polymorphisms and the association of gene expression of IL-10 gene with PTB."	" . . . IL- 10 gene expression is lower in cases as compared to controls and also an association of IL-10 . . . polymorphism with PTB was seen."	1, 2	NR
Paquette 2018	" . . . to characterize the transcriptome in whole blood leukocytes and peripheral monocytes of women undergoing spontaneous preterm labour compared to healthy pregnant women who subsequently delivered at full term."	" . . . identified transcriptomic changes associated with sPTL in maternal WB and PM . . . , filling a critical gap in our understanding of transcriptional regulation of labor induction."	1, 2	>80 % (small sample size does not permit precise estimate)
Paquette 2019	" . . . to identify differences in miRNA expression within whole blood (WB) and peripheral monocytes (PM) . . . of women undergoing sPTL compared with non-labouring controls . . . "	" . . . highlighted miRNA-mediated transcriptional regulatory networks of sPTL-associated genes in monocytes and whole blood, which are involved in important biological pathways, including interleukin signalling"	1, 2	NR
Pawelczyk 2010	" . . . to investigate whether the expression of TLR4 in maternal white blood cells in patients with idiopathic preterm labor is significantly elevated."	1.″ . . . a significant . . . increase in TLR4 mRNA expression in women undergoing spontaneous preterm labor compared with pregnant controls."2.″ . . . elevated TLR4 expression within peripheral WBCs may serve as a useful marker for PTL."	1, 2	60.9%
PrearoMoco 2018	" . . . to evaluate the gene and protein expression of TLR-2 and TLR-4 in maternal neutrophils from women in preterm labor."	"TLR-4 expression in maternal neutrophils is associated with spontaneous preterm labor."	1, 2	NR
Stock 2015	" . . . to measure . . . mRNA coding cytokines in the maternal blood and examine whether they were increased in association with chorioamnionitis at delivery."	"Measuring circulating proinflammatory mRNA in women with PPROM may distinguish those with chorioamnionitis from those without, in turn providing better targeted therapies and appropriate timing of delivery."	1, 2	NA
Tiwari 2016	" . . . delineating the association of differential modulation of progesterone receptor pathway and downstream effectors in the pathogenesis of preterm delivery and outcome."	" . . . sharp downregulation in PR expression is associated with PTD susceptibility, lower gestational period . . . . The PR downstream effector PIBF was also found to be downregulated in PTD, and is associated with gestational period . . . "	1, 2	NR
Truong 2017	" . . . investigated whether oxygen tension alters the exosome release and miRNA profile from extravillous trophoblast (EVT) cells,	1.″ . . . identified a set of unique miRNAs in exosomes . . . isolated from the circulation of mothers at early gestation, who later developed PE and SPTB." 2.″ . . . aberrant exosomal signalling by placental cells is a common	1, 2	NR

Table 2 (*Continued*)

Reference	Primary objective	Major finding	Group	Sensitivity estimate
	modifying their bioactivity on endothelial cells (EC)."	aetiological factor in pregnancy complications . . . and is therefore a clinically relevant biomarker of pregnancy complications."		
Tsai 2017	" . . . compared the individual expression levels of miR-346 and miR-582−3p in three specimen types, including maternal peripheral blood, . . . and further evaluated their association with multiple adverse obstetric outcomes, including . . . , preterm delivery, . . . "	" . . . aberrant miR-346 and miR-582−3p expression level in pregnancy was associated with multiple maternal and fetal complications. Their differential expression in maternal blood, . . . could be potential biomarkers or therapeutic targets for adverse obstetric outcomes"	1, 2	NR
Tyagi 2016	" . . . to investigate the association of OCPs with mRNA expression of TNF-α gene, and gene-gene interaction between TNF-α and COX-2 genes in women who delivered preterm."	" Environmental factors . . . may be associated with inflammatory events showing gene-environment interaction in PTB cases." " . . . may be used as a model to explore the aetiology of idiopathic PTB cases and may be considered for the prognosis of adverse reproductive outcomes."	1, 2	NR
Whitehead 2013a	" . . . examined whether abundance of hypoxia-induced mRNA in the maternal circulation correlates with the degree of fetal hypoxia in utero"	" Abundance of mRNAs coding hypoxia-induced genes circulating in maternal blood strongly correlates with degree of fetal hypoxia/ acidemia."	1, 2	NR
Whitehead 2013b	" . . . examine the expression of a panel of hypoxia induced miRNAs in the maternal blood when the fetus was exposed to: 1) acute hypoxia during labour and 2) chronic hypoxia associated with fetal growth restriction"	" . . . elevation in hypoxia-induced miRNAs in both acute and chronic fetal hypoxia that may be a promising approach to clinically assess fetal hypoxic status in-utero."	1, 2	NR
Whitehead 2013c	"To examine whether mRNA circulating in maternal blood coding genes regulating fetal growth are differentially expressed in (1) severe preterm fetal growth restriction (FGR) and (2) at 28 weeks' gestation in pregnancies destined to develop FGR at term"	"Measuring mRNA coding growth genes in maternal blood may detect unsuspected severe preterm FGR already present in utero, and predict term FGR when measured at 28 weeks' gestation."	1, 2, (3)	NR
Whitehead 2013d	"To determine whether the intrinsic apoptosis pathway is differentially expressed in placenta and maternal blood in severe preterm fetal growth restriction (FGR) and pre-eclampsia (PE), and to examine whether circulating RNA in maternal blood may be potential biomarkers."	"In severe early onset FGR . . . increased expression of genes regulating intrinsic apoptosis in both the placenta and maternal blood. Circulating RNA regulating placenta apoptosis may be used to develop noninvasive novel biomarkers for FGR"	1, 2	NR
Whitehead 2013e	" . . . whether placental specific mRNA transcripts in maternal blood reflect changes in expression in the placental transcriptome and their potential as a novel class of biomarker for FGR."	"There is global differential expression of placental specific mRNA in the maternal blood in pregnancies complicated by severe preterm FGR."	1, 2	NR
Winger 2017	" . . . investigated the capacity of first trimester peripheral blood mononuclear cell (PBMC) microRNA to determine risk of spontaneous preterm birth among pregnant women."	"Quantification of first trimester peripheral blood PBMC MicroRNA may provide sensitive and specific prediction of spontaneous preterm birth in pregnant women."	1, 2, (3)	100 % (early sPTB, EGA < 34) 86 % (late sPTB, CAVE EGA here 34 < 38 weeks)
Wommack 2018	" . . . to 1) investigate cluster-wide associations of pregnancy specific miRNA with length of gestation and birth outcomes and 2) examine whether differences in coordinated expression of circulating miRNA were associated with PTB."	" . . . findings suggest that groups of miRNAs from common chromosomal clusters, rather than individual miRNAs, operate as co-regulated groups of signaling molecules to coordinate length of gestation and infant outcomes."	1, 2	NR
Yuan 2009	1.″ . . . to characterize peripheral blood leukocyte activation during human term and preterm labour." 2.″Additionally, we quantified leukocyte cytokine mRNA production, . . . "	1.″Expression levels of MCP-1 (CCL-2), IL-1b and IL-8 (CXCL8) were significantly greater in labouring women compared with non-labouring women." 2.″There was no effect of gestation on any expression of any of these genes."	1, 2	NR
Zhong 2005	" . . . measured CRH mRNA . . . in women with preterm labor."	" . . . suggest that analysis of circulatory fetal nucleic acids may assist obstetricians in identifying pregnant women with an increased	2	NR

Table 2 (*Continued*)

Reference	Primary objective	Major finding	Group	Sensitivity estimate
		risk of preterm labor who do indeed deliver prematurely"		

Summaries of primary objectives and major finding were quoted from the extracted studies and numbered when multiple objectives and/or findings were reported.
Group allocation: (i) contributes to identifying factors leading to PTB (Group 1), (ii) allows to predict PTB or PTL (Group 2) and if such a prediction is achievable, (iii) at what gestational age a transcriptome screening should be done (Group 3).
Estimation of sensitivity was extracted from papers in which authors attempted and reported a test sensitivity.
Abbreviations: EGA, estimated gestational age; NA, not applicable; NR, not reported, PTB, preterm birth; PTL, preterm labour; sPTB, spontaneous preterm birth; T2, second trimester; T3, third trimester.

gene expression analysis is apparent in this set of publications, considering that the maximum sample size chosen was 1,118 for assessing one target [31], compared to the minimum sample size of 18 for a NGS approach that led to the identification of 52 uniquely expressed miRNAs [30].

Analysis of a small number of targets limits the validity of an inference on a particular molecular pathway. Likely a panel of biomarkers is more sensitive and specific for prediction when compared to a single RNA target. For coding RNA, direct downstream effector functions of the generated proteins are well known. However, non-coding RNA, such as miRNAs, play a role in RNA silencing and post-transcriptional regulation of gene expression. An inference on the exact effector function in the case of miRNAs is not always possible as one miRNA can exert regulatory effects on multiple targets and conversely, one mRNA target can be regulated by multiple miRNAs [46]. Even if the molecular function is poorly understood, the comparisons of miRNA signatures in term pregnancies to PTBs and sPTB in particular, would allow identification of distinct gene expression to inform on risk assessment.

Adequate calculation of test sensitivities was often hindered by small sample sizes. For example the 100 % sensitivity to predict early sPTB (<34 weeks) reported by Winger *et al.* is based on only seven cases [37] and the authors underscore the preliminary nature of their results. Thirty-three papers asserted potential for gene expression analysis to be of predictive value. Considering this overwhelming number, it seems likely that a molecular signature with some predictive value for PTB exists.

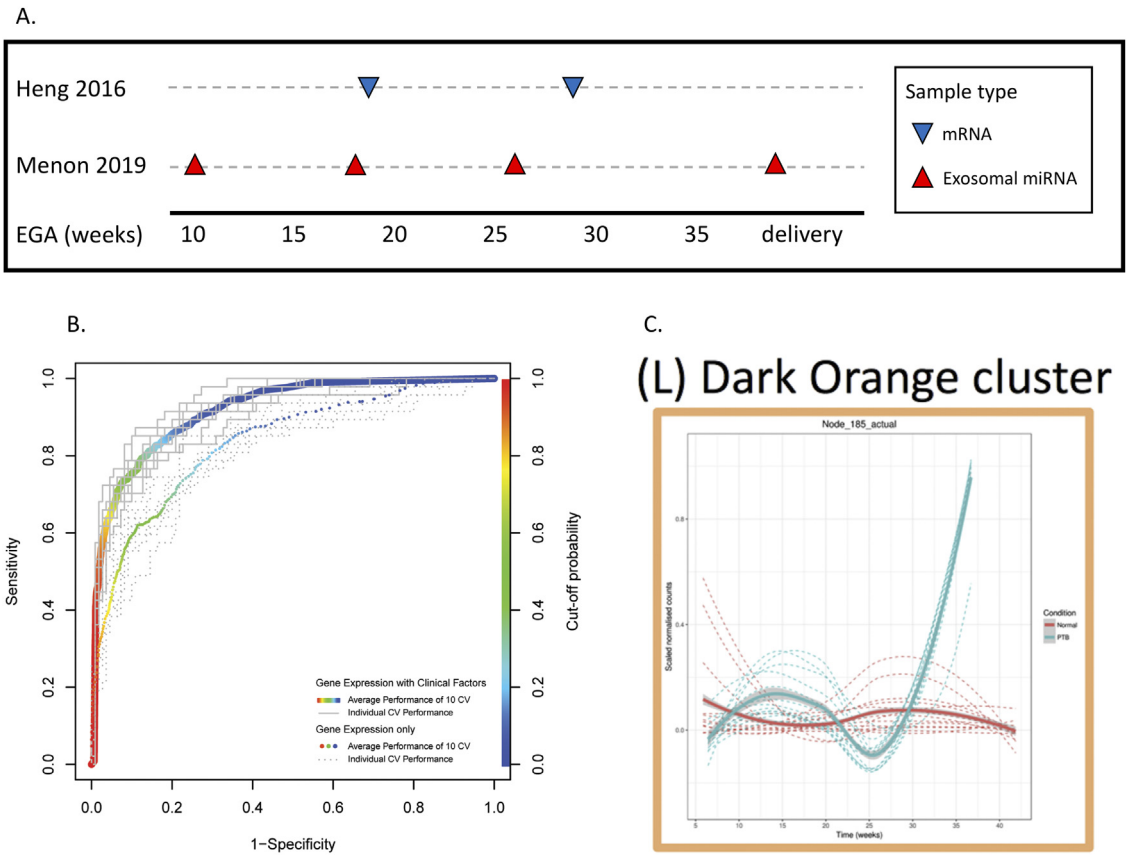

Fig. 3. Studies with predefined, longitudinal sampling that investigated PTB.
(A) Sampling timepoints of Heng *et al.* [32] and Menon *et al.* [33], and the respective RNA sample type. **(B)** Area under receiver operator characteristics curve for a multivariated model associated with PTB constructed by the gene expression fold change from T1 to T2 with clinical factors (solid lines) and without clinical factors (dotted lines) (Heng *et al.*, 2016). **(C)** Linear mixed modelling of statistically significant miRNAs that change across gestation when comparing normal to PTB pregnancies after hierarchical clustering analysis using Euclidean distance. Within the panels, red indicates normal pregnancies whereas blue indicates PTB pregnancies. Dark orange cluster chosen as representation (Menon *et al.*, 2019).

Current evidence on the timing of assessments is limited; most papers assessed gene expression at a single timepoint often at the time of PTL. Blood samples at prespecified timepoints were taken in only four studies. These concluded that gene expression profiling as early as ≤20 weeks EGA, provide putative biomarkers for the prediction of sPTB [30,37,38,47].

Heng *et al.* [32] and Menon *el al.* [33] chose to assess transcriptome signatures of pregnant women at multiple, prespecified timepoints in pregnancy. A longitudinal experimental design is more suitable to inform on the ideal timing to assess deviation from the physiological trajectory and predict PTB. However, as the number of sampling timepoints was only two [32] and four [33], respectively, and the RNA targets differed (mRNA vs. miRNA), these results were not comparable. Fig. 3 provides an overview of these sampling timepoints, illustrates applied methodologies and selected major findings of Heng *et al.* [32] and Menon *et al.* [33], suggesting that transcriptome sampling of maternal blood may indeed be of value in the risk assessment of PTB. The richest sampling schedule was reported by Ngo *et al.* who followed pregnant women longitudinally and took blood weekly [35]. This cohort was a subgroup that was established to assess predictability of EGA by gene expression profiling. However, no women experienced a PTB in this subgroup. In the same study, two other groups of women were assessed for PTB, but blood was only taken at one timepoint prior to PTB.

The overall methodical differences in studies that included only one sampling timepoint does not permit a well-informed estimation on what an appropriate EGA for gene expression assessment to predict PTB would be. Nevertheless, some evidence suggests that routine assessment of blood-derived transcript signatures in early pregnancy supports identification of pregnant women with an increased risk of PTB. Considering that sPTB seems to follow an inheritable disposition, and that a previous PTB increases the risk of a subsequent occurrence, it is astonishing that, to date, no pre-conceptive screening test based on transcriptome assessment has been investigated to assess the risk of a PTB.

If a routine test could identify a pregnant woman with an increased risk for PTB, adequate interventions could be planned prior to onset of PTL. At present, *symptomatic* interventions (e.g. hormone supplementation, cervical cerclage, patient education, etc.) and adequate preparation (e.g. transfer of the pregnant women to specialized preterm birth prevention clinics and/or hospital with specialized neonatal intensive care units) are the best strategies to address an increased preterm birth risk. Whether blocking pathways associated with preterm birth has potential to prevent preterm birth remains to be determined. While interventions that target one specific gene or gene product maybe feasible, unfavourable adverse events may not permit its use. To provide an example: immunomodulators have favourable effects as they may ameliorate excessive immune activation, but, on the other hand, they impair immune function which may increase susceptibility to infection.

Lastly, data were shared by nine authors in public repositories (Table A6). This practise is encouraged to ensure reproducibility, standardization of methods and to increase transparency as it allows other researchers to replicate results and interpret these from a different angle.

5. Conclusion

To date, the potential of *maternal blood transcriptome profiling* for assessing preterm birth risk or aetiology is not used routinely, and hence, remains under appreciated. Although our data, obtained from a systematic review of the existing literature, provide a broader outlook, it has revealed a paucity in standardisation, and profound methodological differences across studies and settings that preclude any useful comparisons. There is a pressing need for clear definitions, standardised protocols and systematic reporting, to identify applicable and reproducible predictors of PTB.

The lack of prospectively followed longitudinal cohorts, with multiple sampling timepoints throughout pregnancy, is particularly striking. An ideal study design should ensure early detection of distinct transcriptome signatures preceding PTB and evaluate less expensive methodologies, compared to e.g. proteomic or metabolomics approaches. WHO recommends a minimum of eight ANC contacts with health care providers, starting in the first trimester – these timepoints could be utilised for prospective observational research to establish transcriptome trajectories predictive of PTB via fingerpick sampling. Adequate and accurate risk prediction models should be developed to inform pregnant women and health care providers about an increased risk of PTB, to guide targeted interventions, and provide a basis for interventional approaches for all countries worldwide.

Funding

No specific funding was obtained for this systematic review.

CRediT authorship contribution statement

Tobias Brummaier: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Basirudeen Syed Ahamed Kabeer:** Data curation, Investigation, Methodology, Writing - review & editing. **Damien Chaussabel:** Methodology, Supervision, Validation, Writing - review & editing. **Jürg Utzinger:** Methodology, Supervision, Validation, Writing - review & editing. **Rose McGready:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing. **Daniel H. Paris:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We would like to take the opportunity and acknowledge the work of all researchers whose research contributed to this systematic review.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eurox.2020.100118.

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