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The transformative potential of an integrative approach to pregnancy

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ABSTRACT

Background: Complex traits typically involve diverse biological pathways and are shaped by numerous genetic and environmental factors. Pregnancy-associated traits and pathologies are further complicated by extensive communication across multiple tissues in two individuals, interactions between two genomes—maternal and fetal—that obscure causal variants and lead to genetic conflict, and rapid evolution of pregnancy-associated traits across mammals and in the human lineage. Given the multifaceted complexity of human pregnancy, integrative approaches that synthesize diverse data types and analyses harbor tremendous promise to identify the genetic architecture and environmental influences underlying pregnancy-associated traits and pathologies.

Methods: We review current research that addresses the extreme complexities of traits and pathologies associated with human pregnancy.

Results: We find that successful efforts to address the many complexities of pregnancy-associated traits and pathologies often harness the power of many and diverse types of data, including genome-wide association studies, evolutionary analyses, multi-tissue transcriptomic profiles, and environmental conditions.

Conclusion: We propose that understanding of pregnancy and its pathologies will be accelerated by computational platforms that provide easy access to integrated data and analyses. By simplifying the integration of diverse data, such platforms will provide a comprehensive synthesis that transcends many of the inherent challenges present in studies of pregnancy.

1. Pregnancy is an ensemble of complex traits subject to substantial genetic and environmental variation

A highly-interconnected network of physiological, cellular, and molecular pathways supports the development of a healthy fetus by maintaining homeostasis through pregnancy despite variation in maternal diet, stress, medical care, and other factors. When genetic and/or environmental variation of this network cannot be buffered to maintain a healthy state, complications arise. Like diseases of other complex traits, the most common complications of pregnancy – preeclampsia (PE), spontaneous preterm birth (sPTB), preterm premature rupture of membranes (PPROM), intrauterine growth restriction (IUGR), and spontaneous recurrent pregnancy loss (RPL) – involve multiple genetic loci and environmental factors [1–5].

Understanding the genetic basis of such complex traits is challenging. For example, although many pregnancy-related traits and pathologies, such as birth timing [1,6], birth weight [6,7], and propensity to develop PE [8], have substantial heritabilities, they are likely governed by numerous genetic variants with small effect sizes and that epistatically interact with each other [2]. Furthermore, pregnancy-related traits are also influenced, to varying degrees, by multiple environmental factors. For example, gestational diabetes [9,10], PE [10,11], and sPTB [9,10,12] are well known for their association with maternal obesity, and sPTB may also be associated with certain environmental exposures, such as bisphenol A [13]. Similarly, chronic and acute stress is thought to reduce birth weight and alter methylation levels of genes involved in the hypothalamic–pituitary–adrenocortical (HPA) axis in the placenta,

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cord blood, and maternal blood [14].

Further complicating matters is that the genetic and environmental factors underlying pregnancy-associated traits and diseases do not act independently; rather, they exhibit gene by environment (GxE) effects, where the pathological phenotype is only observed with specific combinations of genetic variants and environmental conditions. For example, PPROM is often associated with inflammation due to bacterial infection, but recent studies argue that the fetal genotype also influences susceptibility [15]. Specifically, human fetuses with a null *SIGLEC14* genotype were more likely to be born prematurely, but only in conjunction with Group B *Streptococcus* (GBS) infection; in its absence, the *SIGLEC14* null variant did not appear to influence prematurity.

But what makes pregnancy-associated traits and diseases extremely, or maybe even singularly, complex is that they involve three additional dimensions. The first dimension of complexity is associated with the fact that pregnancy-associated traits require coordination and communication across many different tissues and organs in two individuals: the mother and the fetus. The interplay of tissues and organs from two individuals creates many of the distinctive complexities of pregnancy including immunosuppression, entwined physiology (respiration and metabolism), and shared endocrinology. For example, the production of progesterone, which maintains gestation in most placental mammals, must successfully shift from the ovary to the placenta [16]. Similarly, primary human trophoblasts have been shown to release exosomes containing microRNAs, proteins, and phospholipids with antiviral properties, facilitating communication between maternal and fetal tissues [17,18].

The second dimension of complexity in pregnancy is that it involves multiple genomes (maternal, paternal, and fetal), which gives rise to the potential for conflicts of interest over parental investment [19,20]. Parent-of-origin effects on gene expression or genomic imprinting, for example, may have evolved as a result of differences in the consequences of resource investment for paternally and maternally-derived alleles [21,22]. When females mate more than once and offspring are half-sibs, paternally-derived alleles in the fetus may be evolutionarily favored to sequester more resources than optimal from the mother's perspective, favoring imprinting of the maternal allele [23,24].

The third and final dimension of complexity is that of rapid evolutionary change. Pregnancy and its associated tissues evolve rapidly in mammals [25–28], and the placenta is arguably the most diverse mammalian organ [29]. Out of this history of rapid evolutionary change emerged human pregnancy, which is distinctive in its own right [28], as a consequence of several evolutionary events and processes spanning the course of mammalian evolution, including the existence of genetic conflict [30], the primate-specific expansion of cranial size [31], and the human-specific evolution of bipedalism [32].

The importance of considering these additional dimensions of complexity is apparent in hypotheses proposed to explain puzzling facts of human pregnancy. Birth in humans appears to occur sooner than would be expected, given development of the neonate. A much larger fraction of brain growth occurs postnatally in humans than in any other primate. Why? The “obstetrical dilemma” (OD) hypothesis aims to explain gestation length based on two observations unique to human pregnancy – labor that poses risks to both mother and fetus, as well as birth at a point when fetal brain size is only 30% of adult size. The OD hypothesis holds that bipedal locomotion and large cranial capacity, both of which evolved in recent human history, act in opposing ways on the human pelvis, with the result being selection for shortened gestation lengths that preclude cranial expansion beyond pelvic capacity [23,24,33–38]. An alternative hypothesis, known as the “energetic and metabolic constraints on fetal growth and gestation” (EGG) hypothesis, aims to explain gestation length by invoking physiological limitations to metabolic provisioning *in utero*. Here, the primary controlling factor is physiological limits to the transfer of energy and metabolites between the mother and an encephalized fetus [39]. At some point, it is simply more efficient to transfer resources outside of the womb than within.

As these scenarios make clear, neither the genetic basis of pregnancy-associated traits and pathologies nor the proximate or ultimate hypotheses that explain them can be adequately understood from a single experimental approach, data source, or perspective. The history of efforts to decipher the genetic basis of a wide variety of complex traits and diseases offers numerous examples of the perils associated with reliance on a single experimental vantage point [40–42], and the complexities of pregnancy only amplify the need for integrative approaches that combine multiple data types, approaches, or model systems [25,43,44]. In this review, we examine some of the complexities that make human pregnancy-associated traits and pathologies unique and synthesize recent progress in integrative efforts to understand their genetic and environmental dimensions.

2. Integrating multiple maternal and fetal tissues

Pregnancy is singular among human processes in involving coordination of many different tissues and organs from two individuals (Fig. 1), including maternal pregnancy-specific (e.g., decidua, myometrium, and cervix), maternal non-specific (e.g., immune system, metabolism, and endocrine system), and fetal (e.g., lungs, adrenal glands, and fetal membranes). These interactions are responsible for many of pregnancy's unique physiological features, such as immune system modulation [45], entwined respiration and metabolism [46], and shared endocrinology (e.g., progesterone production). True for most placental mammals, the classic example of coordination between maternal and fetal tissues is ensuring that progesterone – a key hormone required for maintenance of gestation – is continuously produced during pregnancy even though the underlying tissue responsible for its production shifts from the ovary to the placenta [16].

The placenta is the nexus of this network of communicating tissues and dedicates a large fraction of its energy budget to secretion and coordination of maternal and fetal needs [47], while also providing the functions of the kidney, the lungs, and the liver for the fetus. Much of this communication occurs locally at the maternal-fetal interface and appears to have evolved early during the evolution of placental mammals by regulatory rewiring of the cAMP signaling pathway in endometrial stromal cells to facilitate decidualization and implantation [48–50]. There are many examples of the importance of communication at the maternal-fetal interface. For example, interaction between maternal immune cells and trophoblasts modulates macrophage inflammatory responses in human pregnancy, which in turn may play a role in implantation and proliferation [51]. Later in gestation, remodeling of the spiral arteries requires successful communication between maternal endothelium and migrating interstitial trophoblasts, which involves both chemotaxis and shifts in cytokine production [52]. Surprising recent work has demonstrated that placental secretion of microRNAs in exosomes at this interface appears to directly increase the resistance of maternal and fetal cell types against viruses implicated in perinatal infections by boosting autophagy [18,53,54].

In addition to local effects at the apposition of maternal-fetal tissues, inter-tissue communication also affects non-adjacent maternal and fetal tissues during gestation. For example, placental production of the neurotransmitter serotonin is crucial

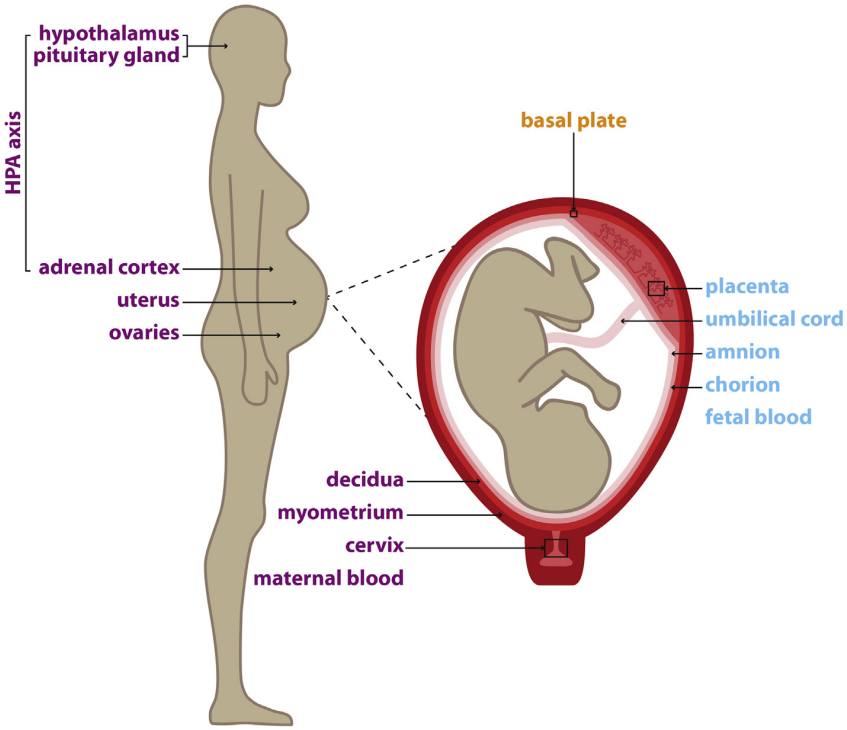

Fig. 1. Pregnancy uniquely requires communication and coordination across multiple tissues in two individuals. Multiple maternal tissues (purple) and fetal tissues (blue) as well as tissues comprised of both maternal and fetal cells (orange) must interact to facilitate a healthy pregnancy. The placenta serves as the nexus of communication that links multiple tissues in the mother and fetus both locally and at a distance. For example, interactions between NK cells in the decidua and fetal trophoblast cells in the placenta shape the degree of placental invasiveness and rate of the exchange of nutrients and oxygen. Similarly, the hypothalamic–pituitary–adrenocortical (HPA) axis communicates maternal and fetal stress levels across multiple tissues through cortisol shared through blood exchange in the placenta.

for embryonic brain development [55,56]. The placenta also influences maternal physiology by secreting high levels of corticotrophin releasing hormone (*CRH*), which leads to higher levels of maternal cortisol, and may be involved in post-partum depression through desensitization to endogenous maternal *CRH* [57]. Medical interventions making use of these communication pathways can potentially be exploited for restoring proper signaling and growth in pathological conditions. Case in point, one recent study has identified synthetic peptides that target liposomes to the maternofetal interface, which restored proper signaling and appropriate placental growth by delivery of *IGF2* in a fetal growth restriction mouse model [58]. This important study provides a proof of principle that, in a mouse model, it is possible to take advance of selective binding at the maternofetal interface for the targeted delivery of therapeutics that improve pregnancy outcome.

Pregnancy thus involves complex crosstalk between multiple interdependent tissues. A comprehensive understanding of adverse pregnancy outcomes is unlikely to emerge from an approach that atomizes pregnancy-associated processes into discrete units of function. More promising are approaches that account for the numerous organs and tissues involved plus all of their interactions in both healthy and pathological states. However, the challenge is that in most cases, sampling of all relevant tissues has not been possible. For example, a recent meta-analysis of 93 global transcriptomic studies across 9 gestational tissues and 29 clinical subtypes showed the paucity of studies that capture multiple tissues with the same clinical phenotype (e.g., many data sets for placental gene expression during PE, but few samples from other tissues) [59]. Even if focus is restricted to examination of single tissues, an additional challenge arises in that independently obtained samples from the same tissue type and clinical subtype show substantial heterogeneity in their gene expression profiles, with only minimal replication of significant genes across studies. This heterogeneity has both technical and biological explanations. Some of the heterogeneity stems from differences in tissue sampling protocols and from differences in the interaction with tissues that were not collected concurrently. Additionally, recent studies have shown that placental gene expression patterns cluster into distinct molecular categories that correlate with maternal symptomology [60,61]. This clustering has also shown improved homogeneity in placental gene expression and illustrates the need for improved molecular phenotyping and experimental design that takes these clusters into account. Nevertheless, much of the observed diversity likely reflects genuine heterogeneity in gene expression within and among individuals, which is evident in global gene expression profiles of placental tissue obtained from healthy pregnancies [62] as well as in comparisons of transcriptional profiles of placental cell types [63].

The extent and magnitude of the observed interactions necessitates the simultaneous sample collection and analysis of multiple tissues, rendering data collection and integration across tissues one of the pressing challenges for understanding pregnancy. An alternative, complementary approach to the simultaneous examination of multiple tissues is *in vitro* reconstruction of tissue interactions, which may become possible as placenta-on-a-chip technology matures [64,65]. A recent study by Blundell et al. [65] demonstrated a model of the human placental barrier using trophoblasts and endothelial cells that accurately reproduces the formation of microvilli and the syncytialization of trophoblasts, and matches the glucose transfer rate of perfused *ex vivo* human placentas.

Furthermore, cell lines of endometrial stromal cells [66], decidual cells [50], and myometrial cells [67] are available, which may lead to more comprehensive pregnancy-on-a-chip approaches. New data from single-cell transcriptomes will also help to detect the effects of local tissue-tissue interactions [63]. Such advances in *in vitro* technology will greatly aid the capture of multiple different data types (e.g., gene expression, protein abundance, phosphorylation, etc.) from interacting tissues simultaneously and in an integrated fashion, which can be used to generate models of tissue interactions. Predictions from such models could in turn be validated through *in vivo* collection of the same data types from the same tissues.

3. Integrating maternal and fetal genomes

The involvement of both the maternal and the fetal genome in pregnancy complicates its study in three related ways. First, a maternal allele can impact pregnancy through either phenotypic expression in the fetus if transmitted or in the mother as either the untransmitted or transmitted maternal allele (Fig. 2). Second, as described above, the phenotypic impact of a fetal allele can vary depending on whether it is maternally or paternally derived, a phenomenon known as parent-of-origin effects associated with genomically imprinted genes [68]. Third, and as a consequence of these two, natural selection may act on alleles differently depending on which genome they find themselves in (maternal or fetal) or which they stem from (maternal or paternal). Therefore, alleles may increase the frequency of their transmission to the next generation by different strategies – improving maternal, fetal, or paternal fitness – which can give rise to genetic conflict since reproductive and/or survival success is different for each organism involved [21].

The complexities inherent in analyses of parental and fetal genetics can be illustrated by considering the well-established correlation of maternal height with gestational age at birth and fetal size [69–74]. Is this correlation causal? If yes, is it driven by phenotypic expression of alleles in the mother or the fetus? To address these questions, Zhang and colleagues [75] used a novel genetic analysis method to disentangle the causal influence of maternal phenotype and genetics from fetal genetics on gestation outcomes. They found that birth length and birth weight were significantly associated with maternal transmitted haplotype whereas gestational age was significantly associated with the maternal non-transmitted haplotype. On the basis of these results, Zhang and colleagues inferred that fetal genetics drives the association between maternal height and fetal growth, but that maternal height, rather than fetal genetics, drives gestational age at birth. This implies that physical and anatomical constraints may contribute to birth timing, as suggested by the OD hypothesis.

Parent-of-origin effects are typically associated with genomic imprinting, where expression of a gene in the fetus or extraembryonic tissue, including the placenta, is only from either the maternally or the paternally inherited allele. Thus, depending on the parent-of-origin, specific variants will be expressed and their effects will be accentuated, whereas other variants won't and their effects will be obscured. Although imprinting was thought to affect most tissues relatively consistently, a recent study revealed a new class of imprinted genes that are specific to the placenta and exhibit paternal allelic expression [76]. These placenta-specific imprinted genes suggest a novel mechanism of imprinting, as these loci are not methylated, the typical mechanism of imprinting, in either sperm or embryonic stem cells [76].

Genomic imprinting and gene expression only from either the maternally or the paternally inherited allele likely arose due to a tug-of-war in fetal resource allocation between the genomes [21,22]; specifically, whereas the selectively favored *fetal* genetic

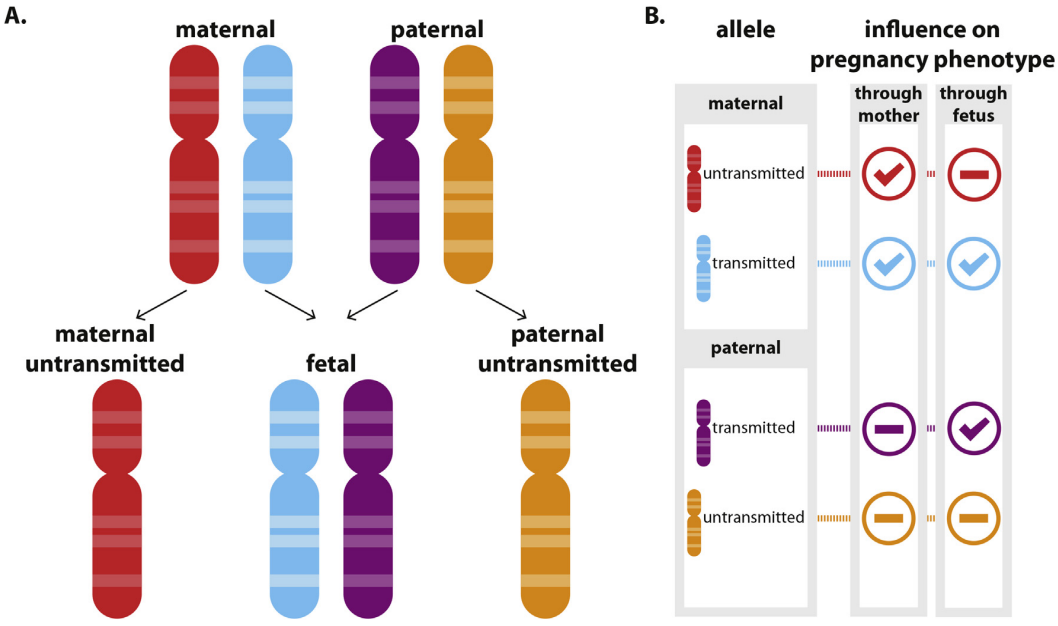

Fig. 2. Involvement of three genomes complicates genetic analyses aimed at linking genes with pregnancy-associated phenotypes. Unlike genetic analyses of most other traits, studies of pregnancy typically require genotyping both mother and fetus, since variants can influence pregnancy through impacting either the maternal or the fetal phenotype or both. Specifically, non-transmitted maternal alleles (red) affect pregnancy only through impact on maternal phenotype; transmitted maternal alleles (blue) can potentially affect pregnancy through impact on maternal and/or fetal phenotype; transmitted paternal alleles (purple) only impact pregnancy through fetal phenotype; finally, non-transmitted paternal alleles (orange) do not affect pregnancy directly. Genetic conflict arises when natural selection differentially favors alleles when they are maternally or paternally expressed, which may contribute to pathologies of pregnancy, including preeclampsia.

variants will be those that optimize resource allocation to the fetus, the selectively favored *maternal* genetic variants will be those that optimize resource allocation across both the current pregnancy but also future ones [23,24]. Where these two optima are in conflict, the kinship theory predicts that maternally-inherited allelic expression (paternal imprinting) will tend to favor slower fetal growth whereas paternally-inherited allelic expression (maternal imprinting) will tend to favor increased fetal growth [22]. Experimental evidence from functional studies of several imprinted genes provide empirical support for these predictions. For example, in mice, the imprinted *Ipl* gene is maternally expressed in both the placenta and the yolk sac, and functions to restrain placental growth and size [77]. In contrast, the imprinted *Igf2* gene is paternally expressed in the mouse placenta, and functions to increase the supply of maternal nutrients to the fetus, augmenting fetal growth [78].

Genetic conflict between maternal, fetal, and paternal genomes has many possible phenotypic outcomes, but two of the most clinically significant, low fetal birth weight and PE, are closely related and mediated by interactions between the maternal immune system and the placental trophoblasts. It might be expected that fetal birth weight is optimized by natural selection, as a healthy weight is associated with increased perinatal survival [79] and reduces the risk of a wide range of adverse adult outcomes (e.g., cognitive development and function, chronic health conditions, disability) [80,81]. Unrestricted fetal growth is undesirable since very large babies also have increased morbidity and mortality. Thus, stabilizing selection acts on birth weight to maintain this balance [82]. However, the maternal genome is favored to optimize resource allocation across multiple offspring [21]. One illustration of this parent-offspring conflict and its influence in fetal birth weight regulation comes from the well-studied interaction between two loci that contain highly polymorphic gene clusters: the *KIR* locus, expressed in maternal natural killer cells, and the *HLA* locus, expressed in implanting placental trophoblasts. A recent study has shown that when a mother has a *KIR* haplotype containing the *KIR2DS1* receptor gene, fetal birth weight increases when the fetus paternally inherits the *HLA-C2* ligand genotype but not when the same allele is maternally inherited [83]. This combination of a maternal genotype and a paternally-inherited fetal genotype has a substantial effect on birth weight, increasing it by almost 10% (~250 g), which is strongly suggestive of *HLA-C2* favoring fetal growth and fitness when paternally-inherited.

An outcome of substantial mismatch in the relative influence of maternal and fetal variants is increased morbidity and/or mortality for both the mother and fetus [84]. Evolutionary biologists have long argued that some pathologies of pregnancy stem precisely from situations in which maternal and fetal reproductive success are not aligned [21,85]. For example, risk for PE is thought to start when the placenta is insufficiently perfused due to inadequate invasion of the decidua by trophoblasts, which leads to increased fetal signals of distress and higher blood pressure [86]. At the genetic level, mismatch occurs when a fetus inherits the paternal *HLA-C2* genotype but the mother has a *KIR AA* genotype since the interaction of these two loci in this specific combination has been shown to increase risk for PE [87]. Interestingly, protective *KIR B* variants have evolved independently in Sub-Saharan African and European populations and are found at different locations within the *KIR* gene cluster [88].

Processes that mediate genetic conflict, including immune interactions and imprinting, introduce unique selective pressures on the maternal, fetal, and paternal genomes. Both the OD and the EGG hypothesis point to the possibility of ongoing genetic conflict between maternal and fetal alleles. Although the OD and EGG hypotheses have been presented as alternative explanations for human fetal development and birth timing, the two views may be complementary and reinforcing. Under both hypotheses, the peak growth of an energy-demanding fetal brain coincides with birth and imposes a substantial maternal cost in energy and risk, which may be compensated for by boosting long-term cognitive development and, presumably, fetal fitness [81,89]. Long-term stability of gestation length may be the result of a compromise between individually optimal maternal, paternal, and fetal fitness. However, the conflict between genomes also plays out on shorter time-scales as illustrated by differences in gestation length and fetal maturity among African, Asian, and European populations [90]. Understanding either timescale requires the integration of maternal and fetal phenotype with maternal and fetal genetic variants and an understanding of evolutionary dynamics.

Genetic conflict in pregnancy appears to lead to faster evolutionary rates across mammals and may lead to the divergence of many pregnancy processes between humans and common model organisms. A recent integrative study found that genes enriched for placental expression evolve faster than genes enriched for expression in most other tissues [25]. Why is this so? Conflict between maternal and fetal genomes has been proposed to result in the “Red Queen” effect, where both genomes must continually evolve to maintain balance [84]. Some of the conflict is thought to be mediated by genomic imprinting [91]. For example, fixation of a new variant of a paternally imprinted gene favoring fetal growth may be countered by fixation of a variant of a maternally imprinted gene restraining fetal growth (and vice versa); repeated cycles of fixations of variants influencing fetal growth would result in acceleration of evolutionary substitution rates in the genes involved, a hallmark of the “Red Queen” effect at the molecular level. Although early examination of the *Igf2* gene and its receptor *Igfr2* as well as of a small number of other imprinted genes in a few mammals did not show evidence of evolutionary rate acceleration [92], a more recent study that examined a broader set of imprinted genes inferred that imprinted genes show increased evolutionary rates relative to non-imprinted genes, consistent with the expectation that genetic conflict results in “Red Queen” effects [26]. Furthermore, the set of human imprinted genes differs substantially from the set of mouse imprinted genes, which suggests ongoing turnover of the proximal mechanisms mediating long-term genetic conflicts [76].

4. Integrating function and evolution of pregnancy

Multiple pregnancy-associated traits evolve rapidly (Fig. 3). For example, the placenta is highly variable across mammals, showing tremendous diversity in structure, shape, invasiveness [27,93]. Along the human lineage, some pregnancy-associated traits may have evolved in conjunction with the rapid evolution of greater cranial capacity and bipedalism, resulting in faster evolutionary rates than would be expected based on genetic conflict alone. Interestingly, one recent study argued that humans may have evolved genetic architecture that allows pelvic size and cranial capacity to co-vary [94]. Women with large heads give birth to babies with larger than average heads, which would seemingly lead to extreme cases of the obstetric dilemma and mortality; however, pelvic size co-varies with cranial size, greatly reducing the risk. Strong selection for this co-variation is presumably less likely in other primates that do not face the OD, although we are unaware of experiments that explicitly test this hypothesis.

A consequence of the fast evolutionary pace of change in mammalian pregnancy is that it is more challenging to predict whether traits observed in one species are also likely to be observed in another [27,95]. For example, it has been commonly assumed that humans are unique in giving birth to “babies born alive before

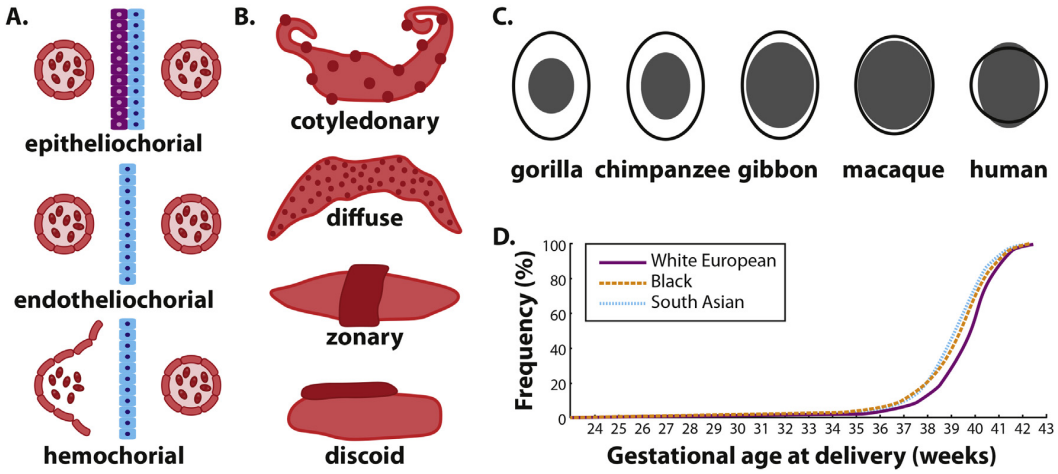

Fig. 3. Pregnancy evolves rapidly across mammals, primates, and human populations. A) Variation in mammalian placentation and fetal access to the maternal bloodstream. This variation can be broadly categorized into three commonly observed modes: epitheliochorial (minimally invasive), endotheliochorial (intermediate), and hemochorial (very invasive). Maternal uterine epithelium (purple) is present only in epitheliochorial placentae; thus, the fetal chorion (blue) is the only epithelium in both endotheliochorial and hemochorial placentae. In hemochorial placentae, the maternal endothelium of blood vessels (pink) is eroded, leading to the formation of blood sinuses (red). As with many pregnancy traits, placentation does not follow a simple evolutionary pattern; rather, varying levels of invasiveness have repeatedly evolved throughout mammals. B) Likewise, the shape of the placenta (pink) and its areas of contact (red) with the underlying uterus varies widely across mammals and includes cotyledonary, diffuse, zonary, and discoid morphologies. C) Primates vary in the relative sizes of the maternal pelvis (open oval) and the fetal cranium (filled oval) at the time of birth, with both macaques and humans sharing a tight fit that appears to have evolved independently in each of the two lineages. Redrawn from Refs. [23,24]. D) Human populations have diverged in gestation length with populations of European ancestry (purple) delayed about one week relative to populations with African (orange) or South Asian (blue) ancestry. Redrawn from Ref. [90].

37 weeks of pregnancy are completed", the definition of PTB given by the World Health Organization [96]. Although this definition is obviously specific to our species, generalizing the human-based definition of PTB as 'parturition prior to 92.5% (37/40 weeks) completed gestation' and applying to mammals in general show that substantial variation in the length of gestation is widespread across mammals and that human gestation length is very similar to what would be expected of a mammal of our body mass [97]. Nevertheless, the dramatic long-term negative effects of preterm birth have not been reported extensively in other species. The rate of human fetal brain expansion peaks at the time of normal parturition and infants are born in a secondarily altricial state, unable to care for themselves. Other primates experience peak rates of brain development much earlier and are born precociously [98–100], which Phillips et al. suggest reduces the fitness consequences for earlier birth [97]. Thus, humans may be unique relative to other mammals due primarily to the timing and importance of brain development, but not with respect to variation in gestation length. More generally, by disentangling of studies of genetic contributors to gestation timing from studies of the medical consequences of premature birth across mammals, the possibility emerges that some mammal models may be useful for the study of gestation timing, while others may be useful for the study of specific PTB-associated pathologies. For example, it could be argued that bats, which have gestation lengths much longer than other mammals of similar size, may be great models for understanding the genetics of gestation timing, even though they may be poor models for studying the pathology of PTB, as prematurely born bats are not known to exhibit any of the pathologies associated with human preterm birth [97,101,102].

The rapid evolution of pregnancy makes the integration of evolutionary analyses and functional data critical to translating discoveries from one species to another, e.g., from the mouse to humans [95]. The first step toward this goal is understanding how the biological systems of pregnancy have diverged between two species. One illustrative example is a recent study that integrated diverse types of data, including gene expression from multiple species, multiple genomic signatures (e.g., CpG island density, sequence conservation, and nucleotide substitution rates), histone modification, copy number variation, TF binding motifs, and ChIP-seq, in order to understand how placental development and gene expression evolve quickly [103]. This integrated analysis revealed that cell lineage-specific enhancers from endogenous retroviruses from the RLTR13D5 family led to substantial divergence of gene regulation in trophoblast stem cells between mouse and rats during 25 million years of separation, a time interval substantially smaller than the 80 million year one demarcating the divergence of rodents and primates [103]. Interestingly, other integrative 'omics analyses have pointed to the importance of transposable elements (TEs) in the evolution of pregnancy [104,105]. For example, a cross-species integration of myometrial RNA-Seq data showed that ancient TEs have been coopted into hormone-responsive regulatory elements coordinating uterine gene expression [104]. Thus, DNA sequences derived from ancient repetitive elements such as endogenous retroviruses and TEs appear to have been repeated coopted into the regulatory landscape of several different tissues associated with mammalian pregnancy.

The rapid evolution of gestation may make clinical translation of animal models more difficult, but it can also be exploited to identify candidate genes based on those evolutionary differences [25,106–109]. For example, a recent study identified over 1000 mammalian genes that repeatedly experienced selection (i.e., accelerated protein sequence evolution) during the evolution of reduced placental invasiveness in other mammals [107]. These genes significantly overlap with genes known to be involved in disorders of pregnancy, particularly PE, and include several associated via GWAS analyses (e.g., *F5*, a coagulation factor; *IL6*, an inflammatory cytokine; and *APOE*, an apolipoprotein) as well as several that are differentially expressed in PE relative to normal gestation (e.g., *S100A8*, a calcium-binding protein involved in inflammatory and immune responses; *CD97*, an adhesion G protein-coupled receptor; and *FLT1*, a vascular endothelial growth factor

receptor). By further integrating evolutionary analyses that predict phenotypic impact of mutations and known genetic and physical interactions, this unique study also narrowed down the original list of candidate genes likely involved in the three independent evolutionary transitions towards reduced placental invasion to 199 genes, all of which are novel candidates for involvement in PE.

Some aspects of pregnancy, such as birth timing [90], evolve so quickly that they have even diverged between modern human populations, raising the possibility that searching for genes exhibiting strong population differentiation may be a fruitful approach to identify gene candidates [108,110]. For example, the leptin gene (*LEP*), which has been previously associated with PE [111–113], shows strong differentiation between human populations [110]. The same is true for the reductase *DHCR7*, which has been associated with both melanoma and preterm birth [108,114].

5. Accelerating integration to facilitate a dynamic understanding of pregnancy

Typically, integrative studies use either multiple data types in a single tissue or the same data type across multiple tissues [42]. In an excellent recent example illustrating the potential power of the first approach, Chu and colleagues integrated high-throughput microRNA, mRNA, and protein expression data to infer an integrated regulatory network that responds to oxidative stress in human placental trophoblast cells [115]. Similarly, another recent study integrated previous GWAS data on PTB with genes differentially expressed between term and preterm samples of myometrial tissue to identify a significant association between parts of two transcription factor networks and PTB [116]. Examples of the second approach are meta-analyses that synthesize knowledge from a single data type across multiple studies investigating different tissues to identify commonalities as well as gaps in current knowledge. One recent comprehensive meta-analysis [59] of genome-scale gene expression studies found a substantial mismatch between currently available data sets related to various pathologies of pregnancy and their worldwide incidence, identifying tissues and pathology combinations (e.g., placenta and sPTB) that are relatively understudied. Specifically, although only 30% of PTB cases are medically indicated, 76% of global gene expression research focuses on these cases. In contrast, 45% of PTB cases occur spontaneously and only 18% of global gene expression research focuses on this major contributor to PTB incidence. Finally, this meta-analysis also revealed that replication of results across studies of the same tissue and pathology is very low, with only 23/10,993 unique genetic elements replicated 10 or more times across 134 studies, reflecting both the high heterogeneity of global gene expression profiles across cell types [63], individuals [62], and populations [62], as well as variability in the design and implementation of pregnancy-related gene expression analyses in general.

As the pace of research accelerates, systematic data integration is moving beyond single publications. For example, Uzun and colleagues have developed dbPTB, which systematically collects genes reported to be associated with preterm birth and is regularly updated, creating a dynamic synthesis of genetic associations [117]. Comprehensive resources like these can provide the foundation for additional analyses that incorporate other kinds of data. For example, integration of preterm birth-related genes reported in dbPTB with data from a previous GWAS study that failed to identify any genome-wide significant candidates allowed for the identification of several pathways (e.g., regulation of blood pressure, smooth muscle contraction, and general metabolism) potentially involved in PTB but missed by the GWAS study alone [118].

Although necessary for advancing our understanding of pregnancy and its pathologies, integration of data is a challenge for many reasons. First, data sets relevant to pregnancy can be difficult to obtain. Existing curation of pregnancy literature has focused more on reporting individual candidate genes (e.g., dbPTB) rather than genome-scale data sets. A meta-analysis of transcriptomes [59] has shown that many papers report genome scale data sets that are not readily available through standard repositories like the Gene Expression Omnibus (GEO), a behavior that is objectionable by present-day standards of practice [119]. Second, re-analysis and quality control of genome scale data sets often require substantial domain-specific knowledge as well as considerable computational expertise. For example, even though RNA-seq data sets can be distilled down to per-gene expression levels (e.g. read counts), a simplification and compression of orders of magnitude, original data sets can involve multiple samples may consist of terabytes of raw data that require substantial computational time. Similarly, integration of multiple types of data requires expertise that may not be readily available in many research labs. Finally, once the raw data has been distilled, integrating data effectively using appropriate statistics, algorithms, and data visualizations requires additional expertise and perspective (Fig. 4).

Open discussion at a recent NIH meeting for the Human Placenta Project [120] highlighted the importance of making data publicly available and providing the bioinformatics tools needed to analyze it. Fortunately, research supporting the integration of pregnancy data and analyses is accelerating. For example, ImmPort (immport.org) is a new platform that makes very large raw datasets easily available for analysis and integration [121]. Adoption of ImmPort by the pregnancy research community will ensure rapid access to the most recent datasets, including ones that are otherwise impractical for individual labs to host; a recent data release (March 2016) included a very large case-control microbiome study of preterm birth [122], which has over 4000 individual metagenomics samples. In addition, following a recent public request for feedback on how to integrate placental images from various technologies and molecular data with physiology and anatomy [123], the NIH is in the process of developing a comprehensive electronic placental atlas tool.

Other databases with more general biological data applicable to the study of pregnancy and its pathologies include Protein Atlas [124], GTeX [125], OMIM [126], and similar resources with information about 'omics in the context of general expression patterns or disease associations. Of note, however, is the limited tissues, phenotypes, and species annotated in each of these resources. For example, although Protein Atlas contains dense information on placental FPKM and genes exhibiting placental-specific expression patterns, these data stem only from healthy, term placentas. Similarly, GTeX contains gene expression information for reproductive tissues like uterus, ovary, and cervix, but lacks data for placenta. Therefore, all of these data are important when considering an integrative approach to pregnancy and its pathologies, but must be utilized as supplementary to other, more targeted 'omics data.

One example of a database designed to facilitate integrative pregnancy research is one developed by the authors, GEneSTATION (Fig. 5A; genestation.org), which provides access to a wide variety of pre-processed data for on-the-fly exploration across multiple data types, species, and human populations [127]. Datasets include gene and protein expression for multiple tissues and pregnancy phenotypes, gene ages and evolutionary rates, allele frequencies across populations, as well as functional annotations and can be analyzed with novel tools like SynTHy (Synthesis and Testing of Hypotheses, Fig. 5B), a framework for the aggregation and cross-filtering of candidate genes according to user-determined filters applied to GEneSTATION data, or integRATE, a desirability function-based data integration software that prioritizes candidate genes in terms of their weight of evidence across relevant research.

Fig. 4. The multi-faceted complexities of pregnancy require integration of multiple types and sources of data. Pregnancy involves two individuals with two distinct genomes (maternal: light red/light green, fetal: dark red/dark green), multiple tissues, and rapid evolution. Each layer of complexity corresponds to a different type of data and successful integration requires careful attention not only to phenotype and tissue, but also to a variety of molecular and evolutionary modulators. Ideally, several different types of 'omics data from the same mothers and babies can be collected across tissues and intersected with relevant evolutionary and functional data to better illuminate the presumably complex pathways leading to pregnancy pathologies.

As the amount and diversity of 'omics data associated with pregnancy phenotypes and pathologies continues to increase, it will be important to keep in mind that numerous computational methods exist for the integration of 'omics data including genomic variation analysis, domain knowledge-guided analysis, concatenation-based integration, transformation-based integration, and model-based integration [for a comprehensive review, see Ref. [128]]. These tools aim to predict phenotypic traits, identify biomarkers, and illuminate genetic underpinnings of complex diseases, like PTB and other pregnancy-associated pathologies. However, the majority of these methods requires the availability of multiple 'omics data types from the same patient cohort and functionally validated genes known to be involved in a given pathology for model training; in our view, generation of such multi-omics data sets from the same set of patients should be a priority for future research.

6. Perspective

In this review, we have outlined the complexities associated with the genetic dissection of pregnancy traits and pathologies and presented several different integrative approaches on how can these can be overcome. Going forward, we believe that integrative approaches will yield the greatest understanding of pregnancy-associated phenotypes when they are joined with research platforms that enable pregnancy researchers from multiple fields to readily access diverse types of pregnancy-related data, quickly obtain and examine syntheses of current data, and efficiently explore the connections between their own data and the existing syntheses. Such platforms will increase the pace and efficiency of discovery by reducing effort duplication in basic data processing, quickly identifying datasets relevant to specific questions, translating knowledge from model organisms into humans and vice versa through the integration of an evolutionary perspective and

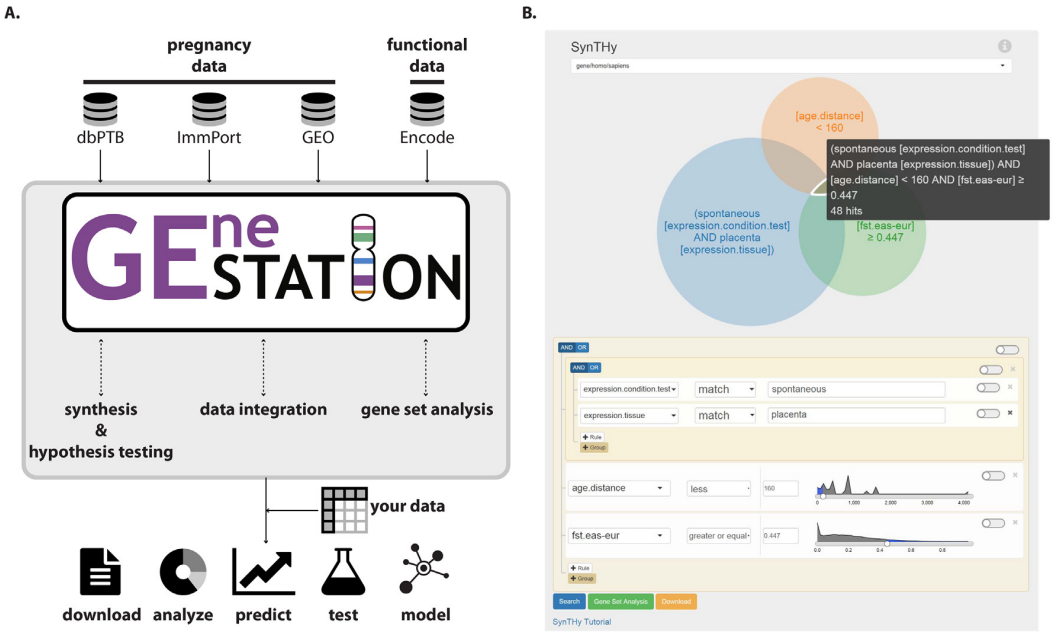

Fig. 5. GEneSTATION is a new platform for integration of diverse *'omics* and evolutionary data types that facilitates rapid analyses. **A**. GEneSTATION integrates pregnancy-specific data from a number of public sources with functional data that is not specific to disease. The platform provides users with rapid, simple tools for generating and testing hypotheses, integrating data, and analyzing sets of candidate genes using both existing data and user submitted data. Results from all analyses can be easily downloaded and used to prioritize candidate genes for functional involvement in a trait or pathology. **B**. One example visual analytical tool from GEneSTATION, SynTHy, allows integration of many data types, including differential gene expression in pregnancy tissues for various pathologies, gene ages, and genetic differences between populations (F_{ST}). In the example analysis, the user has selected genes that are differentially expressed in spontaneous preterm birth in the placenta, arose in the lineage leading to placental mammals, and associated with genetic variants that show substantially different frequencies between South Asian and European population ($F_{ST} \geq 0.447$).

analyses, and providing scientists with intuitive tools that help them confidently and effortlessly integrate data and analyses from multiple disciplines.

Given the multi-faceted complexities of pregnancy, it is encouraging that the agencies funding pregnancy research have shifted their funding strategies to prioritize approaches that integrate new approaches with existing data and knowledge. In light of the substantial challenges involved in reanalyzing a wide variety of data (request from authors, validation/curation, and basic analysis), future integrative approaches will require data and analysis platforms that make diverse kinds of existing pregnancy data readily available and interoperable, along with algorithms to easily integrate and interpret new data.

The diverse complexities of pregnancy make integrative approaches a necessary part of all future pregnancy research. Rapid discovery and improved clinical care will be the fruit of community efforts to improve data access, to facilitate powerful multi-disciplinary analyses by non-experts, and to develop platforms that promote collaboration across disciplines.

Conflict of interest statement

The authors declare no conflict of interest.

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References

[1] R. Romero, S.K. Dey, S.J. Fisher, Preterm labor: one syndrome, many causes, Science 345 (2014) 760–765, http://dx.doi.org/10.1126/science.1251816.
[2] R. Romero, H. Kuivaniemi, G. Tromp, J. Olson, The design, execution, and interpretation of genetic association studies to decipher complex diseases, Am. J. Obstetrics Gynecol. 187 (2002) 1299–1312.
[3] K.A. Pennington, J.M. Schlitt, D.L. Jackson, L.C. Schulz, D.J. Schust, Pre-eclampsia: multiple approaches for a multifactorial disease, Dis. Model Mech. 5 (2012) 9–18, http://dx.doi.org/10.1242/dmm.008516.
[4] N. Hendrix, V. Berghella, Non-placental causes of intrauterine growth restriction, Semin. Perinatol. 32 (2008) 161–165, http://dx.doi.org/10.1053/j.semperi.2008.02.004.
[5] C. Lengyel, L.J. Muglia, M. Pavličev, Genetics of Preterm Birth, eLS. (n.d.). http://dx.doi.org/10.1002/9780470015902.a0025448.
[6] B. Clausson, P. Lichtenstein, S. Cnattingius, Genetic influence on birthweight and gestational length determined by studies in offspring of twins, Bjog 107 (2000) 375–381.
[7] P. Magnus, H.K. Gjessing, A. Skrondal, R. Skjaerven, Paternal contribution to birth weight, J. Epidemiol. Community Health 55 (2001) 873–877, http://dx.doi.org/10.1136/jech.55.12.873.
[8] H. Salonen Ros, P. Lichtenstein, L. Lipworth, S. Cnattingius, Genetic effects on the liability of developing pre-eclampsia and gestational hypertension, Am. J. Med. Genet. 91 (2000) 256–260.
[9] M.R. Torloni, A.P. Betrán, B.L. Horta, M.U. Nakamura, A.N. Atallah, A.F. Moron, et al., Prepregnancy BMI and the risk of gestational diabetes: a systematic review of the literature with meta-analysis, Obes. Rev. 10 (2009) 194–203, http://dx.doi.org/10.1111/j.1467-789X.2008.00541.x.
[10] J. Marchi, M. Berg, A. Dencker, E.K. Olander, C. Begley, Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews, Obes. Rev. 16 (2015) 621–638, http://dx.doi.org/10.1111/obr.12288.
[11] Z. Wang, P. Wang, H. Liu, X. He, J. Zhang, H. Yan, et al., Maternal adiposity as an independent risk factor for pre-eclampsia: a meta-analysis of prospective cohort studies, Obes. Rev. 14 (2013) 508–521, http://dx.doi.org/10.1111/obr.12025.
[12] C.S. Lengyel, S. Ehrlich, J.D. Iams, L.J. Muglia, E.A. DeFranco, Effect of modifiable risk factors on preterm birth: a population based-cohort, Matern. Child. Health J. 21 (2016) 777–785, http://dx.doi.org/10.1007/s10995-016-2169-8.
[13] C.J. Patel, T. Yang, Z. Hu, Q. Wen, J. Sung, Y.Y. El-Sayed, et al., Investigation of maternal environmental exposures in association with self-reported preterm

birth, Reprod. Toxicol. 45 (2014) 1–7, http://dx.doi.org/10.1016/j.reprotox.2013.12.005.
[14] D.A. Kertes, H.S. Kamin, D.A. Hughes, N.C. Rodney, S. Bhatt, C.J. Mulligan, Prenatal maternal stress predicts methylation of genes regulating the hypothalamic–pituitary–adrenocortical system in mothers and newborns in the democratic republic of Congo, Child. Dev. 87 (2016) 61–72, http://dx.doi.org/10.1111/cdev.12487.
[15] S.R. Ali, J.J. Fong, A.F. Carlin, T.D. Busch, R. Linden, T. Angata, et al., Siglec-5 and Siglec-14 are polymorphic paired receptors that modulate neutrophil and amnion signaling responses to group B Streptococcus, J. Exp. Med. 211 (2014) 1231–1242, http://dx.doi.org/10.1084/jem.20131853.
[16] A.I. Csapo, M.O. Pulkkinen, B. Ruttner, J.P. Sauvage, W.G. Wiest, The significance of the human corpus luteum in pregnancy maintenance. I. Preliminary studies, Am. J. Obstetrics Gynecol. 112 (1972) 1061–1067.
[17] Y. Ouyang, J.F. Mouillet, C.B. Coyne, Y. Sadovsky, Review: placenta-specific microRNAs in exosomes - good things come in nano-packages, Placenta (35 Suppl) (2014) S69–S73, http://dx.doi.org/10.1016/j.placenta.2013.11.002.
[18] E. Delorme-Axford, R.B. Donker, J.-F. Mouillet, T. Chu, A. Bayer, Y. Ouyang, et al., Human placental trophoblasts confer viral resistance to recipient cells, Pnas 110 (2013) 12048–12053, http://dx.doi.org/10.1073/pnas.1304718110.
[19] M. Ishida, D. Monk, A.J. Duncan, S. Abu-Amero, J. Chong, S.M. Ring, et al., Maternal inheritance of a promoter variant in the imprinted PHLDA2 gene significantly increases birth weight, Am. J. Hum. Genet. 90 (2012) 715–719, http://dx.doi.org/10.1016/j.ajhg.2012.02.021.
[20] G.E. Moore, M. Ishida, C. Demetriou, L. Al-Olabi, L.J. Leon, A.C. Thomas, et al., The role and interaction of imprinted genes in human fetal growth, Phil. Trans. R. Soc. B 370 (2015), http://dx.doi.org/10.1098/rstb.2014.0074, 20140074–20140074.
[21] D. Haig, Genetic conflicts in human-pregnancy, Q. Rev. Biol. 68 (1993) 495–532.
[22] T. Moore, Genomic imprinting in mammalian development: a parental tug-of-war, Trends Genet. 7 (1991) 45–49.
[23] A.H. Schultz, The Life of Primates, 1969.
[24] K. Rosenberg, W. Trevathan, Birth, obstetrics and human evolution, Bjog 109 (2002) 1199–1206.
[25] J. Hirbo, H. Eidem, A. Rokas, P. Abbot, Integrating diverse types of genomic data to identify genes that underlie adverse pregnancy phenotypes, PLoS One 10 (2015) e0144155, http://dx.doi.org/10.1371/journal.pone.0144155.
[26] B. Hutter, M. Bieg, V. Helms, M. Paulsen, Divergence of imprinted genes during mammalian evolution, BMC Evol. Biol. 10 (2010) 116, http://dx.doi.org/10.1186/1471-2148-10-116.
[27] H.W. Mossman, Vertebrate Fetal Membranes: Comparative Ontogeny and Morphology; Evolution; Phylogenetic Significance; Basic Function; Research Opportunities, Macmilian, London, 1987.
[28] P. Abbot, A. Rokas, Mammalian pregnancy, Curr. Biol. 27 (2017) R127–R128, http://dx.doi.org/10.1016/j.cub.2016.10.046.
[29] M. Garratt, J.-M. Gaillard, R.C. Brooks, J.-F. Lemaître, Diversification of the eutherian placenta is associated with changes in the pace of life, Pnas 110 (2013) 7760–7765, http://dx.doi.org/10.1073/pnas.1305018110.
[30] H.G. Spencer, A.G. Clark, M.W. Feldman, Genetic conflicts and the evolutionary origin of genomic imprinting, Trends Ecol. Evol. (Amst.) 14 (1999) 197–201, http://dx.doi.org/10.1016/S0169-5347(98)01556-0.
[31] N. Jeffery, Brain expansion and comparative prenatal ontogeny of the non-hominoid primate cranial base, J. Hum. Evol. 45 (2003) 263–284, http://dx.doi.org/10.1016/j.jhevol.2003.08.002.
[32] D. Schmitt, Insights into the evolution of human bipedalism from experimental studies of humans and other primates, J. Exp. Biol. 206 (2003) 1437–1448.
[33] W.M. Krogman, The scars of human evolution, Sci. Am. 185 (1951) 54–57, http://dx.doi.org/10.1038/scientificamerican1251-54.
[34] S.L. Washburn, Tools and human evolution, Sci. Am. 203 (1960) 62–75, http://dx.doi.org/10.1038/scientificamerican0960-62.
[35] K. Rosenberg, W. Trevathan, Bipedalism and human birth: the obstetrical dilemma revisited, Evol. Anthropol. Issues, News, Rev. 4 (1995) 161–168, http://dx.doi.org/10.1002/evan.1360040506.
[36] A.B. Wittman, L.L. Wall, The evolutionary origins of obstructed labor: bipedalism, encephalization, and the human obstetric dilemma, Obstet. Gynecol. Surv. 62 (2007) 739–748, http://dx.doi.org/10.1097/01.ogx.0000286584.04310.5c.
[37] W. Trevathan, Primate pelvic anatomy and implications for birth, Phil. Trans. R. Soc. B 370 (2015), http://dx.doi.org/10.1098/rstb.2014.0065, 20140065–20140065.
[38] L.T. Gruss, D. Schmitt, The evolution of the human pelvis: changing adaptations to bipedalism, obstetrics and thermoregulation, Phil. Trans. R. Soc. B 370 (2015), http://dx.doi.org/10.1098/rstb.2014.0063, 20140063–20140063.
[39] H.M. Dunsworth, A.G. Warrener, T. Deacon, P.T. Ellison, H. Pontzer, Metabolic hypothesis for human altriciality, Pnas 109 (2012) 15212–15216, http://dx.doi.org/10.1073/pnas.1205282109.
[40] V.K. Mootha, P. Lepage, K. Miller, J. Bunkenborg, M. Reich, M. Hjerrild, et al., Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 605–610, http://dx.doi.org/10.1073/pnas.242716699.
[41] U.D. Akavia, O. Litvin, J. Kim, F. Sanchez-Garcia, D. Kotliar, H.C. Causton, et al., An integrated approach to uncover drivers of cancer, Cell 143 (2010) 1005–1017, http://dx.doi.org/10.1016/j.cell.2010.11.013.
[42] Y. Hasin, M. Seldin, A. Lusis, Multi-omics Approaches to Disease, 2017, pp. 1–15, http://dx.doi.org/10.1186/s13059-017-1215-1.
[43] S. Gracie, C. Pennell, G. Ekman-Ordeberg, S. Lye, J. McManaman, S. Williams, et al., An integrated systems biology approach to the study of preterm birth using "-omic" technology–a guideline for research, BMC Pregnancy Childbirth 11 (2011) 71, http://dx.doi.org/10.1186/1471-2393-11-71.
[44] E.M. Lackritz, C.B. Wilson, A.E. Guttmacher, J.L. Howse, C.M. Engmann, C.E. Rubens, et al., A solution pathway for preterm birth: accelerating a priority research agenda, Lancet Glob. Health 1 (2013) e328–e330, http://dx.doi.org/10.1016/S2214-109X(13)70120-7.
[45] G. Mor, I. Cardenas, Review article: the immune system in pregnancy: a unique complexity, Am. J. Reprod. Immunol. 63 (2010) 425–433, http://dx.doi.org/10.1111/j.1600-0897.2010.00836.x.
[46] J. Mele, S. Muralimanoharan, A. Maloyan, L. Myatt, Impaired mitochondrial function in human placenta with increased maternal adiposity, Am. J. Physiol. Endocrinol. Metab. 307 (2014) E419–E425, http://dx.doi.org/10.1152/ajpendo.00025.2014.
[47] A.M. Carter, Placental oxygen consumption. Part I: in vivo studies—a review, Placenta 21 (2000) S31–S37.
[48] V.J. Lynch, R.D. Leclerc, G. May, G.P. Wagner, Transposon-mediated rewiring of gene regulatory networks contributed to the evolution of pregnancy in mammals, Nat. Genet. 43 (2011) 1154–1159, http://dx.doi.org/10.1038/ng.917.
[49] G.P. Wagner, K. Kin, L. Muglia, M.P. 269, ev, Evolution of mammalian pregnancy and the origin of the decidual stromal cell, Int. J. Dev. Biol. 58 (2014) 117–126, http://dx.doi.org/10.1387/ijdb.130335gw.
[50] K. Kin, M.C. Nnamani, V.J. Lynch, E. Michaelides, G.P. Wagner, Cell-type phylogenetics and the origin of endometrial stromal cells, Cell Rep. 10 (2015) 1398–1409.
[51] S. Fest, P.B. Aldo, V.M. Abrahams, I. Visintin, A. Alvero, R. Chen, et al., Trophoblast–macrophage interactions: a regulatory network for the protection of pregnancy, Am. J. Reprod. Immunol. 57 (2007) 55–66, http://dx.doi.org/10.1111/j.1600-0897.2006.00446.x.
[52] P.B. Aldo, G. Krikun, I. Visintin, C. Lockwood, R. Romero, G. Mor, A novel three-dimensional in vitro system to study trophoblast–endothelium cell interactions, Am. J. Reprod. Immunol. 58 (2007) 98–110, http://dx.doi.org/10.1111/j.1600-0897.2007.00493.x.
[53] E. Delorme-Axford, A. Bayer, Y. Sadovsky, C.B. Coyne, Autophagy as a mechanism of antiviral defense at the maternal–fetal interface, Autophagy 9 (2013) 2173–2174, http://dx.doi.org/10.4161/auto.26558.
[54] A. Bayer, E. Delorme-Axford, C. Sleigher, T.K. Frey, D.W. Trobaugh, W.B. Klimstra, et al., Human trophoblasts confer resistance to viruses implicated in perinatal infection, Am. J. Obstetrics Gynecol. 212 (2015) 71.e1–71.e8.
[55] A. Bonnin, N. Goeden, K. Chen, M.L. Wilson, J. King, J.C. Shih, et al., A transient placental source of serotonin for the fetal forebrain, Nature 472 (2011) 347–350, http://dx.doi.org/10.1038/nature09972.
[56] R. McKay, Developmental biology: remarkable role for the placenta, Nature 472 (2011) 298–299, http://dx.doi.org/10.1038/472298a.
[57] M. Thomson, The physiological roles of placental corticotropin releasing hormone in pregnancy and childbirth, J. Physiol. Biochem. 69 (2013) 559–573, http://dx.doi.org/10.1007/s13105-012-0227-2.
[58] A. King, C. Ndifon, S. Lui, K. Widdows, V.R. Kotamraju, L. Agemy, et al., Tumor-homing peptides as tools for targeted delivery of payloads to the placenta, Sci. Adv. 2 (2016), http://dx.doi.org/10.1126/sciadv.1600349 e1600349–e1600349.
[59] H.R. Eidem, W.E. Ackerman, K.L. McGary, P. Abbot, A. Rokas, Gestational tissue transcriptomics in term and preterm human pregnancies: a systematic review and meta-analysis, BMC Med. Genomics 8 (2015) 27, http://dx.doi.org/10.1186/s12920-015-0099-8.
[60] K. Leavey, S.A. Bainbridge, B.J. Cox, Large scale aggregate microarray analysis reveals three distinct molecular subclasses of human preeclampsia, PLoS One 10 (2015), http://dx.doi.org/10.1371/journal.pone.0116508.
[61] K. Leavey, S.J. Benton, D. Grynspan, J.C. Kingdom, S.A. Bainbridge, B.J. Cox, Unsupervised placental gene expression profiling identifies clinically relevant subclasses of human preeclampsia, Hypertension 68 (2016) 137, http://dx.doi.org/10.1161/HYPERTENSIONAHA.116.07293.
[62] D.A. Hughes, M. Kircher, Z. He, S. Guo, G.L. Fairbrother, C.S. Moreno, et al., Evaluating intra- and inter-individual variation in the human placental transcriptome, Genome Biol. 16 (2015) 54, http://dx.doi.org/10.1186/s13059-015-0627-z.
[63] M. Pavlicev, G.P. Wagner, A.R. Chavan, K. Owens, J. Maziarz, C. Dunn-Fletcher, et al., Single-cell transcriptomics of the human placenta: inferring the cell communication network of the maternal-fetal interface, Genome Res. 27 (2017) 349–361, http://dx.doi.org/10.1101/gr.207597.116.
[64] J.S. Lee, R. Romero, Y.M. Han, H.C. Kim, C.J. Kim, J.-S. Hong, et al., Placenta-on-a-chip: a novel platform to study the biology of the human placenta, J. Maternal-Fetal Neonatal Med. 29 (2015) 1046–1054, http://dx.doi.org/10.3109/14767058.2015.1038518.
[65] C. Blundell, E.R. Tess, A.S.R. Schanzer, C. Coutifaris, E.J. Su, S. Parry, et al., A microphysiological model of the human placental barrier, Lab. Chip 16 (2016) 3065–3073, http://dx.doi.org/10.1039/C6LC00259E.
[66] G. Krikun, G. Mor, A. Alvero, S. Guller, F. Schatz, A novel immortalized human endometrial stromal cell line with normal progestational response, J. Qual.

Technol. 145 (5) (2004) 2291–2296.
[67] J. Fitzgibbon, J.J. Morrison, T.J. Smith, M. O'Brien, Modulation of human uterine smooth muscle cell collagen contractility by thrombin, Y-27632, TNF alpha and indomethacin, Reprod. Biol. Endocrinol. 7 (2009) 2, http://dx.doi.org/10.1186/1477-7827-7-2.
[68] H.A. Lawson, J.M. Cheverud, J.B. Wolf, Genomic imprinting and parent-of-origin effects on complex traits, Nat. Rev. Genet. 14 (2013) 609–617, http://dx.doi.org/10.1038/nrg3543.
[69] C.R.S. Dougherty, A.D. Jones, The determinants of birth weight, Am. J. Obstetrics Gynecol. 144 (1982) 190–200.
[70] P.M. Catalano, J.P. Kirwan, Maternal factors that determine neonatal size and body fat, Curr. Diab Rep. 1 (2001) 71–77, http://dx.doi.org/10.1007/s11892-001-0013-y.
[71] Z. Han, O. Lutsiv, S. Mulla, S.D. McDonald, on behalf of the Knowledge Synthesis Group, Maternal height and the risk of preterm birth and low birth weight: a systematic review and meta-analyses, J. Obstetrics Gynaecol. Can. 34 (2012) 721–746.
[72] O.Y. Addo, A.D. Stein, C.H. Fall, D.P. Gigante, A.M. Guntupalli, B.L. Horta, et al., Maternal height and child growth patterns, J. Pediatr. 163 (2013) 549–554.e1.
[73] K. Myklestad, L.J. Vatten, E.B. Magnussen, K.Å. Salvesen, P.R. Romundstad, Do parental heights influence pregnancy length?: a population-based prospective study, HUNT 2, BMC Pregnancy Childbirth 13 (2013) 33, http://dx.doi.org/10.1186/1471-2393-13-33.
[74] N.-H. Morken, K. Källen, B. Jacobsson, Predicting risk of spontaneous preterm delivery in women with a singleton pregnancy, Paediatr. Perinat. Epidemiol. 28 (2014) 11–22, http://dx.doi.org/10.1111/ppe.12087.
[75] G. Zhang, J. Bacelis, C. Lengyel, K. Teramo, M. Hallman, Ø. Helgeland, et al., Assessing the causal relationship of maternal height on birth size and gestational age at birth: a mendelian randomization analysis, PLoS Med. 12 (2015) e1001865, http://dx.doi.org/10.1371/journal.pmed.1001865.
[76] F. Court, C. Tayama, V. Romanelli, A. Martin Trujillo, I. Iglesias-Platas, K. Okamura, et al., Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment, Genome Res. 24 (2014) 554–569, http://dx.doi.org/10.1101/gr.164913.113.
[77] D. Frank, W. Fortino, L. Clark, R. Musalo, W. Wang, A. Saxena, et al., Placental overgrowth in mice lacking the imprinted gene Ipl, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 7490–7495, http://dx.doi.org/10.1073/pnas.122039999.
[78] M. Constância, M. Hemberger, J. Hughes, W. Dean, A. Ferguson-Smith, R. Fundele, et al., Placental-specific IGF-II is a major modulator of placental and fetal growth, Nature 417 (2002) 945–948, http://dx.doi.org/10.1038/nature00819.
[79] L.V. Valen, R. Weiss, Selection in natural populations V. Indian Rats (Rattus Rattus), Genet. Res. 8 (2009) 261, http://dx.doi.org/10.1017/S0016672300010144.
[80] M. Richards, Birth weight and cognitive function in the British 1946 birth cohort: longitudinal population based study, Bmj 322 (2001) 199–203, http://dx.doi.org/10.1136/bmj.322.7280.199.
[81] D. Figlio, J. Guryan, K. Karbownik, J. Roth, The effects of poor neonatal health on children's cognitive development, Am. Econ. Rev. 104 (2014) 3921–3955.
[82] L. Ulizzi, L. Terrenato, Natural selection associated with birth weight. VI. Towards the end of the stabilizing component, Ann. Hum. Genet. 56 (1992) 113–118.
[83] S.E. Hiby, R. Apps, O. Chazara, L.E. Farrell, P. Magnus, L. Trogstad, et al., Maternal KIR in combination with paternal HLA-C2 regulate human birth weight, J. Immunol. 192 (2014) 5069–5073, http://dx.doi.org/10.4049/jimmunol.1400577.
[84] R. Pijnenborg, L. Vercruysse, M. Hanssens, Fetal-maternal conflict, trophoblast invasion, preeclampsia, and the red queen, Hypertens. Pregnancy 27 (2009) 183–196, http://dx.doi.org/10.1080/10641950701826711.
[85] R.L. Trivers, Parent-offspring conflict, Am. Zoologist 14 (1) (1974) 249–264.
[86] G.J. Burton, A.L. Fowden, The placenta: a multifaceted, transient organ, Phil. Trans. R. Soc. B 370 (2015), http://dx.doi.org/10.1098/rstb.2014.0066, 20140066–20140066.
[87] S.E. Hiby, R. Apps, A.M. Sharkey, L.E. Farrell, L. Gardner, A. Mulder, et al., Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2, J. Clin. Invest 120 (2010) 4102–4110, http://dx.doi.org/10.1172/JCI43998.
[88] A. Nakimuli, O. Chazara, S.E. Hiby, L. Farrell, S. Tukwasibwe, J. Jayaraman, et al., A KIR B centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia, Pnas 112 (2015) 845–850, http://dx.doi.org/10.1073/pnas.1413453112.
[89] S.C. Cunnane, M.A. Crawford, Survival of the fattest: fat babies were the key to evolution of the large human brain, Comp. Biochem. Physiology (2003), http://dx.doi.org/10.1016/S1095-6433(03)00048-5.
[90] M. Scioscia, M.A. Paine, K. Gumaa, C.H. Rodeck, T.W. Rademacher, Release of inositol phosphoglycan P-type by the human placenta following insulin stimulus: a multiple comparison between preeclampsia, intrauterine growth restriction, and gestational hypertension, J. Maternal-Fetal Neonatal Med. 21 (2009) 581–585, http://dx.doi.org/10.1080/14767050802199934.
[91] E. Li, C. Beard, R. Jaenisch, Role for DNA methylation in genomic imprinting, Nature 366 (1993) 362–365, http://dx.doi.org/10.1038/366362a0.
[92] G.T. McVean, L.D. Hurst, Molecular evolution of imprinted genes: no evidence for antagonistic coevolution, Proc. R. Soc. Lond. B Biol. Sci. 264 (1997) 739–746, http://dx.doi.org/10.1098/rspb.1997.0105.
[93] D.E. Wildman, Review: toward an integrated evolutionary understanding of the mammalian placenta, Placenta 32 (Suppl 2) (2011) S142–S145, http://dx.doi.org/10.1016/j.placenta.2011.01.005.
[94] B. Fischer, P. Mitteroecker, Covariation between human pelvis shape, stature, and head size alleviates the obstetric dilemma, Pnas 112 (2015) 5655–5660, http://dx.doi.org/10.1073/pnas.1420325112.
[95] C.K. Ratajczak, J.C. Fay, L.J. Muglia, Preventing preterm birth: the past limitations and new potential of animal models, Dis. Model Mech. 3 (2010) 407–414, http://dx.doi.org/10.1242/dmm.001701.
[96] World Health Organization, Preterm Birth. Fact Sheet No. 363, WHO, Geneva, 2014, p. 2014, http://dx.doi.org/10.1046/j.1469-0705.1998.12050312.x/full.
[97] J.B. Phillips, P. Abbot, A. Rokas, Is preterm birth a human-specific syndrome? Evol. Med. Public Health 2015 (2015) 136–148, http://dx.doi.org/10.1093/emph/eov010.
[98] J. Dobbing, The later growth of the brain and its vulnerability, Pediatrics 53 (1974) 2–6.
[99] J. Dobbing, J. Sands, Comparative aspects of the brain growth spurt, Early Hum. Dev. 3 (1979) 79–83.
[100] T. Sakai, S. Hirata, K. Fuwa, K. Sugama, K. Kusunoki, H. Makishima, et al., Fetal brain development in chimpanzees versus humans, Curr. Biol. 22 (2012) R791–R792, http://dx.doi.org/10.1016/j.cub.2012.06.062.
[101] R.D. Martin, A.M. MacLarnon, Gestation Period, Neonatal Size and Maternal Investment in Placental Mammals, 1985, http://dx.doi.org/10.1038/313220a0.
[102] M. Clauss, M.T. Dittmann, D.W.H. Müller, P. Zerbe, D. Codron, Low scaling of a life history variable: analysing eutherian gestation periods with and without phylogeny-informed statistics, Mamm. Biol. 79 (2014) 9–16, http://dx.doi.org/10.1016/j.mambio.2013.01.002.
[103] E.B. Chuong, M.A.K. Rumi, M.J. Soares, J.C. Baker, Endogenous retroviruses function as species-specific enhancer elements in the placenta, Nat. Genet. 45 (2013) 325–329, http://dx.doi.org/10.1038/ng.2553.
[104] V.J. Lynch, M.C. Nnamani, A. Kapusta, K. Brayer, S.L. Plaza, E.C. Mazur, et al., Ancient transposable elements transformed the uterine regulatory landscape and transcriptome during the evolution of mammalian pregnancy, Cell Rep. 10 (2015) 551–561, http://dx.doi.org/10.1016/j.celrep.2014.12.052.
[105] A. Kapusta, Z. Kronenberg, V.J. Lynch, X. Zhuo, L. Ramsay, G. Bourque, et al., Transposable elements are major contributors to the origin, diversification, and regulation of vertebrate long noncoding RNAs, PLoS Genet. 9 (2013) e1003470, http://dx.doi.org/10.1371/journal.pgen.1003470.
[106] J. Plunkett, S. Doniger, G. Orabona, T. Morgan, R. Haataja, M. Hallman, et al., An evolutionary genomic approach to identify genes involved in human birth timing, PLoS Genet. 7 (2011) e1001365, http://dx.doi.org/10.1371/journal.pgen.1001365.
[107] M.G. Elliot, B.J. Crespi, Genetic recapitulation of human pre-eclampsia risk during convergent evolution of reduced placental invasiveness in eutherian mammals, Phil. Trans. R. Soc. B 370 (2015), http://dx.doi.org/10.1098/rstb.2014.0069, 20140069–20140069.
[108] M. Huang, B.E. Graham, G. Zhang, R. Harder, N. Kodaman, J.H. Moore, et al., Evolutionary triangulation: informing genetic association studies with evolutionary evidence, BioData Min. 9 (2016) 12, http://dx.doi.org/10.1186/s13040-016-0091-7.
[109] E.J. Crosley, M.G. Elliot, J.K. Christians, B.J. Crespi, Placental invasion, pre-eclampsia risk and adaptive molecular evolution at the origin of the great apes: evidence from genome-wide analyses, Placenta 34 (2013) 127–132, http://dx.doi.org/10.1016/j.placenta.2012.12.001.
[110] K.E. Holsinger, B.S. Weir, Genetics in geographically structured populations: defining, estimating and interpreting FST, Nat. Rev. Genet. 10 (2009) 639–650, http://dx.doi.org/10.1038/nrg2611.
[111] Y. Ouyang, H. Chen, H. Chen, H. Chen, Reduced plasma adiponectin and elevated leptin in pre-eclampsia, Int. J. Gynecol. Obstet. 98 (2007) 110–114, http://dx.doi.org/10.1016/j.ijgo.2007.04.021.
[112] H. Mise, N. Sagawa, T. Matsumoto, S. Yura, H. Nanno, H. Itoh, et al., Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia, J. Clin. Endocrinol. Metab. 83 (1998) 3225–3229, http://dx.doi.org/10.1210/jcem.83.9.5117.
[113] J.L. Bartha, R. Romero Carmona, M. Escobar Llompart, R. Comino Delgado, The relationships between leptin and inflammatory cytokines in women with pre-eclampsia, Bjog 108 (2001) 1272–1276, http://dx.doi.org/10.1111/j.1471-0528.2001.00284.x.
[114] E.N.A. Bream, C.R. Leppellere, M.E. Cooper, J.M. Dagle, D.C. Merrill, K. Christensen, et al., Candidate gene linkage approach to identify DNA variants that predispose to preterm birth, Pediatr. Res. 73 (2012) 135–141, http://dx.doi.org/10.1038/pr.2012.166.
[115] T. Chu, J.-F. Mouillet, B.L. Hood, T.P. Conrads, Y. Sadovsky, The assembly of miRNA-mRNA-protein regulatory networks using high-throughput expression data, Bioinformatics 31 (2015) 1780–1787, http://dx.doi.org/10.1093/bioinformatics/btv038.
[116] D. Brubaker, Y. Liu, J. Wang, H. Tan, G. Zhang, B. Jacobsson, et al., Finding lost genes in GWAS via integrative-omics analysis reveals novel sub-networks associated with preterm birth, Hum. Mol. Genet. 25 (2016) 5254–5264, http://dx.doi.org/10.1093/hmg/ddw325.
[117] A. Uzun, A. Laliberte, J. Parker, C. Andrew, E. Winterrowd, S. Sharma, et al., dbPTB: a database for preterm birth, Database 2012 (2012), http://dx.doi.org/10.1093/database/bar069 bar069–bar069.

[118] A. Uzun, A.T. Dewan, S. Istrail, J.F. Padbury, Pathway-based genetic analysis of preterm birth, Genomics 101 (2013) 163–170, http://dx.doi.org/10.1016/j.ygeno.2012.12.005.
[119] T. Barrett, S.E. Wilhite, P. Ledoux, C. Evangelista, I.F. Kim, M. Tomashevsky, et al., NCBI GEO: archive for functional genomics data sets–update, Nucleic Acids Res. 41 (2013) D991–D995, http://dx.doi.org/10.1093/nar/gks1193.
[120] A.E. Guttmacher, C.Y. Spong, The human placenta project: it's time for real time, Am. J. Obstetrics Gynecol. 213 (2015) S3–S5, http://dx.doi.org/10.1016/j.ajog.2015.08.037.
[121] S. Bhattacharya, S. Andorf, L. Gomes, P. Dunn, H. Schaefer, J. Pontius, et al., ImmPort: disseminating data to the public for the future of immunology, Immunol. Res. 58 (2014) 234–239, http://dx.doi.org/10.1007/s12026-014-8516-1.
[122] D.B. DiGiulio, B.J. Callahan, P.J. McMurdie, E.K. Costello, D.J. Lyell, A. Robaczewska, et al., Temporal and spatial variation of the human microbiota during pregnancy, Pnas 112 (2015) 11060–11065, http://dx.doi.org/10.1073/pnas.1502875112.
[123] NOT-HD-15–1005: Request for Information (RFI): An Internet Resource for Placental Research - Placental Atlas Tool, (n.d.)., n.d. http://grants.nih.gov/grants/guide/notice-files/NOT-HD-15-005.html.
[124] M. Uhlén, L. Fagerberg, B.M. Hallström, C. Lindskog, P. Oksvold, A. Mardinoglu, et al., Proteomics. Tissue-based map of the human proteome, Science 347 (2015) 1260419, http://dx.doi.org/10.1126/science.1260419.
[125] GTEx Consortium, The genotype-tissue expression (GTEx) project, Nat. Genet. 45 (2013) 580–585, http://dx.doi.org/10.1038/ng.2653.
[126] J.S. Amberger, C.A. Bocchini, F. Schiettecatte, A.F. Scott, A. Hamosh, OMIM.org: online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders, Nucleic Acids Res. 43 (2015) D789–D798, http://dx.doi.org/10.1093/nar/gku1205.
[127] M. Kim, B.A. Cooper, R. Venkat, J.B. Phillips, H.R. Eidem, J. Hirbo, et al., GEneSTATION 1.0: a synthetic resource of diverse evolutionary and functional genomic data for studying the evolution of pregnancy-associated tissues and phenotypes, Nucleic Acids Res. 44 (2016) D908–D916, http://dx.doi.org/10.1093/nar/gkv1137.
[128] M.D. Ritchie, E.R. Holzinger, R. Li, S.A. Pendergrass, D. Kim, Methods of integrating data to uncover genotype-phenotype interactions, Nat. Rev. Genet. 16 (2015) 85–97, http://dx.doi.org/10.1038/nrg3868.