Stress and Preterm Birth

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Abstract

Each year 15 million babies are born preterm and the global rate of preterm birth (PTB) of >10% is steadily rising. PTB programs life-long complications, including long term motor, cognitive, growth and health problems. A major obstacle in determining the causes of this global problem is that PTB is multifactorial with several different etiologies. These various etiologies ultimately converge on identifiable pathways that are critically influenced by stress. In this review we focus on lifetime and transgenerational aspects of stress related to PTB and discuss possible mechanisms that translate stress and associated allostatic load into the physiological changes that transform the uterus of pregnancy to the uterus of delivery preterm. The concept of allostatic load induced by a severe stressor provides a compelling rationale for the contribution of stress to spontaneous PTB. Although it is challenging to demonstrate a clear causal relationship in humans, PTB was recognized as a consequence of severe maternal distress preconceptionally. For example, adverse childhood experiences increase the risk for PTB.

Being born preterm is the most powerful predictor for a woman to be at higher risk for a PTB of her own. Recent findings in rodents indicate that prenatal stress may generate an epigenetic signature that may be passed on to the offspring, thus enhancing stress sensitivity and interfering with pregnancy maintenance in the next and future generations. Based on this work in animal models, prenatal stress may predispose women, their daughters and granddaughters to greater PTB risk. Epigenetic mechanisms

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may help explain gene x environment interactions that link childhood abuse and expression of genetic risk factors.

We propose that PTB represents a multifactorial condition, which according to the “Two-Hit Hypothesis” can be triggered or worsened by an episode of prenatal stress or a traumatic life event. New cost-effective technologies, including stress assessments and interventions, need to be developed to firstly identify women at high risk of PTB and then treat them in order to delay preterm delivery, prolong pregnancy and thereby improve newborn health outcomes.

**Keywords:** Allostasis, cortisol, preterm birth, stress, transgenerational programming

### Introduction

The publication of the report, *Born Too Soon*, in 2012 by the World Health Association, the March of Dimes and other international sponsors focused worldwide attention on the problem of preterm birth (PTB, birth before 37 completed weeks of pregnancy) (World Health Organization, 2012). Not only were the statistics alarming, that each year 15 million babies are born preterm, 1.1 million of these will die, and the global PTB rate of >10% is rising, but equally concerning was the revelation that there is no jurisdiction anywhere that is exempt from the problem. In a very real sense, PTB is a global health problem.

One of the major factors contributing to this global problem is that PTB is multifactorial with several different etiologies. While these various etiologies ultimately converge on identifiable pathways that transform the uterus of pregnancy to the uterus of delivery, teams of investigators are seeking the initiating causes in order to identify the modifiable risk factors in the hope that suitable prognostics and interventions will ultimately be available to identify those at risk, reduce the incidence of PTB and prolong pregnancy in order to improve newborn health outcomes. The highest rates of PTB, those at 15% or higher, typically occur in low and middle-income countries in sub-Saharan Africa, southern Asia and extend to Indonesia and the Philippines where health status, poverty, poor or uneven nutrition or limited access to care are frequently considered major causes. But this axiom is not consistent as the United States, a high-income country, has one of the highest rates at around 12%. Such information highlights the diversity of the problem and one wonders if indeed there are any common causal threads that cross income, social status, access, ethnic, or cultural lines. Increasingly, stress is being examined as one possible common denominator in the etiology of PTB. In this review we will focus on lifetime and transgenerational aspects of stress related to PTB and identify possible mechanisms that translate allostatic load into the physiological changes that transform the uterus of pregnancy to the uterus of delivery preterm. We will not attempt to cover all the stress and PTB literature nor address acute stress and PTB, rather we will present representative examples from our work and that of others that address the above.
Stress

Stress is a word that is heard everywhere and every day. Yet, it is a very ambiguous term as it means something different to everyone. It is often used in a negative sense, but is stress always bad for you? In general terms, stress can be defined as any challenge – psychological or physical – that threatens or that is perceived to threaten homeostasis (Berhman and Butler, 2006). It encompasses both environmental demands and cognitive and emotional responses, such as stress perception, to those demands. Over the years, scientists have developed various biological and psychological concepts to define stress and the stress response.

Homeostasis. Walter Cannon (1871-1945) developed the concept of homeostasis, based on the milieu intérieur (Claude Bernard), and he used the following concept in defining homeostasis: physiological reactions are coordinated to maintain a steady state or equilibrium in the body (Cannon, 1928, 1929). This is required to sustain life. Homeostasis includes the regulation of body temperature, pH, blood glucose and oxygen tension. Cannon stressed the importance of the autonomic nervous system as a homeostatic control mechanism. Hans Selye (1907-1982) was an endocrinologist and a pioneer in the field of biological stress. He introduced the well-known ‘General Adaptation Syndrome’ (GAS) model in 1936, based on endocrine experiments in mice. The general adaptation syndrome describes the physiological adaptive reactions in the body in response to a stressor (Selye, 1998, Selye and Fortier, 1950):

1. Alarm reaction – ‘Fight or flight’ response: initial reaction stage in the body with activation of the autonomous nervous system and hormonal systems to prepare for ‘action.’
2. Stage of resistance: adaptation stage in which the body actively copes with the stressor and attempts to return to a homeostatic state.
3. Stage of exhaustion: when stress persists beyond the capability of the body to cope, the body becomes exhausted resulting in (permanent) damage to internal organs and increased susceptibility to disease.

According to Selye, GAS is largely dependent on the function of the autonomic nervous system. In his model, a stressor – whether an injury, damaging agent or psychological event – always influences certain tissues directly, followed by systemic damage and defence due to nervous and hormonal mediation. In his search to identify the hormonal mediators of GAS, Selye was the first to describe the role of the hypothalamic-pituitary-adrenocortical axis (HPA axis) in response to a stressor.

The HPA axis. Any type of physical or mental stress in a human can elicit a rapid and greatly enhanced secretion of the main stress hormone, the glucocorticoid (GC) cortisol (or corticosterone in rodents). The physiologic stress response involves the autonomic nervous system, in particular the sympathetic adrenal medullary (SAM) axis and the HPA axis (Guyton and Hall, 2006, Stratakis and Chrousos, 1995). Both systems originate in the brain. The hypothalamus, located in the middle of the base of the brain, is the ‘control center’ of the neuroendocrine system in the body and plays a vital role in maintaining homeostasis. The paraventricular nucleus of the hypothalamus contains neuroendocrine neurons that synthesize and secrete corticotropin-releasing hormone (CRH) (Vale et al., 1981). CRH release from the hypothalamus is under nervous control and influenced by the sleep/wake cycle, by cortisol levels in the blood, and by stress. After secretion, CRH is transported through the portal blood
vessel system of the pituitary stalk to the anterior pituitary gland, where it binds to the corticotropin releasing hormone receptor (CRHR1) on the corticotrope cells. This stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH) stored in corticotrope cells. ACTH is then released into the blood stream and transported to the adrenal cortex of the adrenal glands. ACTH stimulates the synthesis of glucocorticoids, mainly cortisol, from cholesterol via activation of adenylyl cyclase and formation of cAMP in the zona fasciculata of the adrenal cortex (Smith et al., 1982).

Cortisol, or hydrocortisone, is a steroid hormone that acts on the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), but it has a higher affinity for the GR (De Kloet et al., 1998). The GR is present in almost every cell in the body (Lu et al., 2006). When bound to the GR, cortisol has important effects on carbohydrate metabolism, protein metabolism, fat metabolism, and on stress and inflammatory responses (Guyton and Hall, 2006). Cortisol stimulates gluconeogenesis and glycogenesis in the liver while reducing the utilization of glucose by the cells, resulting in an increased blood glucose level in the body. Cortisol has catabolic effects, such as proteolysis and lipolysis, in tissues. The resulting mobilization of amino acids and fatty acids, together with higher blood glucose levels, can be considered as adaptive processes to provide energy substrates for the body, in particular the heart and the brain, to cope with demands. Cortisol also exhibits anti-inflammatory effects. It suppresses leukocyte proliferation and migration, and it inhibits the synthesis of pro-inflammatory cytokines resulting in a suppressed inflammatory response (Elenkov et al., 1999, Wadhwa et al., 2001). Circulating cortisol also inhibits the secretion of ACTH. It exerts its negative feedback effect both at the level of the anterior pituitary gland and the hypothalamus.

The autonomic nervous system via the SAM axis also plays a vital role in the stress response. The catecholamines, epinephrine (adrenaline) and, in much lesser extent, norepinephrine (noradrenaline), are produced by the adrenal medulla through sympathetic stimulation and indirectly through cortisol. Epinephrine is especially important in acute or emergency situations when the sympathetic nervous system is activated – the fight or flight response. Circulating (nor)epinephrine causes vasoconstriction in essentially all blood vessels and stimulates the heart. In addition, epinephrine has a metabolic effect by increasing both plasma glucose and plasma fatty acid concentrations due to enhanced utilization of fat during stress.

Cognitive appraisal and coping. Whereas Cannon and Selye mainly measured the physiological responses to external stressors, it was later thought that external stressors are mediated by the perception of the individual. Therefore Lazarus and Cohen defined a biobehavioural model of the stress response (Lazarus and Cohen, 1977). They stated that when ‘environmental demands, such as chronic stressors, exceed the adaptive capacity of an organism, the result can be psychological and biological changes that may place persons at risk of disease.’ Adding to the complexity of their model, they argued that cognitive appraisal plays a central role (Folkman et al., 1986a, 1986b). Each individual evaluates whether a particular encounter with the environment is relevant to his or her well-being and whether it will be perceived as stressful. In addition, individual coping skills – cognitive and behavioural efforts to manage the internal and external demands – and modifiers of the stress response, such as social support, are important elements in the stress response. For instance, someone with an extensive and adequate support network may well appraise stressors more optimistically or use adaptive coping styles to solve a problem since friends and family are.
available for support. In contrast, people in an abusive environment may have a more negative outlook on the world and this affects their cognitive appraisal of stressors. Adequate social support, adaptive coping skills, resilience and optimism are all moderators of stress and can be seen as ‘protective,’ resulting in decreasing perceived stress and better health outcomes. Conversely, maladaptive coping styles and risky behaviour and lifestyle, such as smoking and alcohol use, may initially seem to reduce perceived stress for many people, however they can actually increase the stress response and lead to negative health outcomes (Dai et al., 2007, Steptoe and Ussher, 2006).

Allostasis and allostatic load. In the late 1980’s, an alternative term to homeostasis was introduced into the stress literature: allostasis. Originally proposed by Sterling and Ever, allostasis is the adaptive process for actively maintaining stability through change (Sterling and Ever, 1988). Allostasis is derived from the Greek ‘allo’, meaning ‘variable’, while ‘stasis’ means ‘stand.’ Therefore, allostasis means ‘stable by being variable,’ and it is a fundamental process supporting homeostasis through which the body can adjust to stressors. Allostasis – extensively described by McEwen – is achieved through the production of mediators of the stress response such as adrenal hormones, inflammatory cytokines and neurotransmitters that help us adapt to new situations and challenges (McEwen, 1998a, 1998b, McEwen and Seeman, 1999, McEwen and Wingfield, 2003). The brain plays a central role in allostasis, by controlling various mechanisms simultaneously. In acute situations, allostasis is beneficial for the body, as it is essential for the body to respond and adapt to stressors. As a result, an effective physiological solution to the threat is achieved. However, when allostasis is prolonged – in chronic or repetitive stress – the autonomic nervous system and HPA axis are repetitively activated and the neuroendocrine and inflammatory adaptive processes now become damaging to the body. For this, McEwen coined the term ‘allostatic load,’ defined as the cumulative results of allostasis. In other words, it comprises the ‘wear and tear’ of allostasis over a lifetime on the body and the brain. In chronic stress, the allostatic load increases as the body attempts to cope with stressors. Thus, the main hormonal mediators, cortisol and epinephrine, that normally maintain homeostasis, now have a negative effect on the body, resulting in the acceleration of disease processes such as cardiovascular disease. In addition, allostatic load over a long period of time might cause the allostatic systems to become exhausted leading to dysregulation of the HPA axis and compensatory responses by other systems. Chronic stress can therefore result in an increase in inflammatory cells and cytokines and increased susceptibility to infection and inflammation (Djuric et al., 2008, Elenkov et al., 1999, McEwen, 1998a).

Preterm Birth

Preterm infants are at greater risk for mortality, developmental delay, adverse health conditions, morbidity, lower quality of life and lost potential than infants born at term (Mikkola et al., 2005). PTB programs life-long complications, including long term motor, cognitive, visual, hearing, behavioural, social-emotional, health, and growth problems (Mikkola et al., 2005), presumably contributing to an individual’s allostatic load. Having a previous preterm delivery or being born preterm are amongst the most powerful predictors known at present for a woman to have a PTB in her current pregnancy (Ekwo et al., 1992,
Goldenberg et al., 2008) – an effect often related to genetics (likely epigenetics), but possibly also to a high allostatic load.

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Long-term health complications, such as increased risk of PTB, may result from adverse perinatal programming by stress and low birth weight (Challis and Smith, 2001, Hobel, 2004, Rich-Edwards and Grizzard, 2005, Wainstock et al., 2013, Zhu et al., 2010). Preterm birth, a leading cause of neonatal morbidity and mortality, is associated with an intrauterine pro-inflammatory state triggered by unknown causes in about 50% of cases. It has been difficult to demonstrate a clear causal relationship in humans (Kramer et al., 2013), although it was noted that PTB risk may be influenced from cumulative effects of lifetime stress (Rich-Edwards and Grizzard, 2005). PTB was recognized as a consequence of severe maternal distress during pregnancy (Hobel, 2004, Zhu et al., 2010) or due to preconceptional factors (Emanuel et al., 1999).

Gestational stress may affect levels of hormones and neuropeptides, including prolactin, progesterone, and oxytocin, which are involved in maintenance of pregnancy and timing of delivery (Arck, 2001, Miller and Riegle, 1985) In animal studies, adverse experience was suggested to compromise the continuation of gestation (Arck, 2001). Cumulative effects of stress over a lifetime seem to be of particular importance to PTB (Rich-Edwards and Grizzard, 2005). Arguably, prenatal stress in the offspring, by permanently altering the cytokine milieu, may predispose to pregnancy complications in later life (Coussons-Read et al., 2012). Such programming of physiological and inflammatory responses in early life may transmit through subsequent generations (Crews et al., 2012). Inter-generational programming of hypothalamic-pituitary-adrenal (HPA) axis activity during pre- and early postnatal development can become a key regulator of adult disease (Kaati et al., 2002, Zucchi et al., 2013) and behaviour (Franklin et al., 2011, Ward et al., 2013, Weiss et al., 2011). Thus, it is reasonable to assume that maternal stress during pregnancy may program physiological responses, PTB risk and birth outcomes across multiple generations. Indeed, PTB risk was noted to propagate through generations (Porter et al., 1997).

Prenatal Stress and Offspring Development

The gestational state is a period of particular vulnerability to stress for both the mother and her offspring. The effects of maternal stress before or during pregnancy have been shown to have considerable adverse effects upon the offspring and examples of this follow.

Exposure of a fetus to GCs during maternal stress is regulated by the placental enzyme 11-β hydroxysteroid dehydrogenase type 2 (11β-HSD2). Circulating levels of physiological GC are much higher in the maternal than in the fetal blood. This gradient is ensured by the 11β-HSD2 which catalyzes the rapid inactivation of GCs to their inert 11-keto forms, thus forming a natural barrier to maternal GCs (Charil et al., 2010, Yang et al., 1990). Nevertheless, the fetoplacental 11β-HSD2 gene is down-regulated by maternal stress (Sarkar et al., 2001) and excess GC levels caused by severe maternal stress may pass the placenta to reach the fetus (Newnham, 2001, Seckl, 2008). Thus, excessive maternal GCs or 11β-HSD2
inhibition are able to modulate the developing fetal HPA axis and its regulation in later life, altering brain development and HPA axis functions throughout the lifetime (Harris and Seckl, 2011).

In the long-term, HPA axis programming by prenatal stress causes a reduction in its negative feedback mechanism to elevate stress sensitivity and generate loss of resilience to adverse challenges. It was suggested that greater stress sensitivity leads to greater vulnerability to neuropsychiatric conditions, including depression, bipolar affective disorder and schizophrenia (Fine et al., 2014, Kleinhaus et al., 2013, Zucchi et al., 2013), as well as metabolic and cardiovascular disease (Boersma et al., 2014, Buchwald et al., 2012, van Dijk et al., 2012, Zucchi et al., 2014).

Stress sensitivity contributes to vulnerability to stress-associated endocrine and immunological manifestations through both excessive catecholamine and GC secretion and through GR resistance (Cohen et al., 2012). The latter in particular increases production of pro-inflammatory cytokines (Cohen et al., 2012), which in turn attenuate 11β-HSD2 enzymatic activity in human placenta (Challis and Smith, 2001, Kossintseva et al., 2006). These and related mechanisms critically determine maternal health and alter offspring brain physiology with life-long consequences (Harris and Seckl, 2011, Schwab, 2009), generating a state of particular vulnerability to PTB.

Immune stress to pregnant Long-Evans rat dams in the form of interleukin (IL)-1β administration from gestational days (GD) 17-21 leads to impaired cognitive performance (less time spent investigating a novel object) in male and female offspring and to affective behaviour in female offspring (Paris et al., 2011). It also reduces utilization of progesterone in the hippocampus of female offspring as defined by conversion to dihydroprogesterone and allopregnanolone (see section on Neuroendocrine mechanisms). Allopregnanolone plays an important role in the development of the central nervous system by promoting neuronal growth early in fetal life and later protecting neural development, promoting cognitive function, attenuating anxiety, and attenuating the oxytocin release apparatus (Chin et al., 2011, Herbison, 2001). Very similar results in terms of impaired offspring cognitive function were obtained when this group substituted restraint stress or chronic unpredictable stressors to pregnant dams for immune stress (Paris and Frye, 2011a, 2011b).

**Prenatal Stress and Transgenerational Programming**

The perinatal period is a time of high vulnerability to environmental influences. As discussed above, maternal stress can influence offspring development and stress responses with consequences potentially lasting to adulthood. This raises the possibility that prenatal stress, or the long-term endocrine, metabolic, immunological or behavioural consequences associated with it, may produce a phenotype that is not confined to the first generation but may propagate to subsequent generations. Transgenerational changes have been reported for many environmental stimuli, with one of the most potent being stress (Crews et al., 2012, Drake et al., 2005, Dygalo et al., 1999).

To date, the most-investigated mechanisms of phenotypic programming by perinatal adversity include altered gestational endocrine milieu, maternal behaviour and transgenerational epigenetic programming (Champagne and Meaney, 2007, Harris and Seckl, 2011, Migicovsky and Kovalchuk, 2011, Zucchi et al., 2012). Recent studies focusing on
transmission through the male germ line in rodents have suggested that altered stress responses and associated emotional traits are caused by ancestral exposure to environmental toxins (Crews et al., 2012) and stressful experiences (Dias and Ressler, 2014, Gapp et al., 2014, Morgan and Bale, 2011). In the maternal lineage, prenatal exposure to endocrine disruptors (Nilsson et al., 2008, Skinner et al., 2013) or to maternal undernutrition in humans (Veenendaal et al., 2013) has been associated with increased metabolic and endocrine disease risk in the offspring.

The function of transgenerational epigenetic programming may assist in preparing the offspring’s progeny to certain postnatal conditions through inherited, acquired epigenetic modifications (Migicovsky and Kovalchuk, 2011, Skinner et al., 2010). However, in the long term perinatal stress may also lead to maladaptive re-programming that promotes pathological processes leading to disease (Babenko et al., 2012, Relton and Davey Smith, 2010). Transgenerational programming occurs when an epigenetic change is incorporated into the germ line escaping the reprogramming process and manifests itself in the absence of the causative agent in the female F3 generation (Crews et al., 2012). It is the F3 generation that allows the unequivocal determination of transgenerational phenotypes and will assist in identifying epigenetic prognostic markers and preventive strategies.

### Stress-Associated PTB Studies

Maternal stress during pregnancy is increasingly recognized as a variable of interest in the etiology of spontaneous PTB, however its contribution to the risk of PTB remains controversial (see Wadhwa et al., 2011 and Chen et al., 2011 for a more comprehensive review of the issues associated with assessing correlations between stress and PTB in women). Studies examining the effect of maternal stress during pregnancy on PTB have shown varied results, partly due to the fact that they have only explored separate stressors and their relationship with PTB. Often, cognitive appraisal of stressors or individual responses were not considered in the studies. Moreover, there is a lack of the use of a comprehensive measure of chronic stress. The concept of allostatic load provides a compelling rationale for the contribution of chronic stress to spontaneous PTB. Therefore, examining the exposure to stressors over a mother’s life course might give a better perspective on the role of maternal stress in the etiology of spontaneous PTB.

### Natural Disasters, Terrorist Attacks and Chemical Disasters

Harville, Xiong and Buekens (2010) published an excellent systematic review of the relationship between several types of natural or man-caused disasters on adverse pregnancy outcomes that we will summarize (Harville et al., 2010). The United Nations defines disaster as “a serious disruption of the functioning of a community or a society involving widespread human, material, economic, or environmental losses and impacts, which exceeds the ability of the affected community or society to cope using its own resources” (http://www.eird.org/wikien/index.php/Glossary). The authors also defined disaster as ‘a discrete precipitating event, such as a hurricane, earthquake, terrorist attack, or chemical spill.
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because such events share some similar qualities, but differ as well. The former revolves around the degree of exposure to the disaster and the consequent issues of variable access to food and health care, whereas the latter is that each disaster and its ‘environmental exposure’ is unique (Baum and Fleming, 1993, Bongers et al., 2008, Callaghan et al., 2007, Kusuda et al., 1995, Norris and Uhl, 1993, Sapir, 1993).

World Trade Centre, September 11, 2001. Fetuses of women exposed to the environmental effects of 9/11 had an increased risk of intrauterine growth retardation at birth (Berkowitz et al., 2003). Infants born to women residing near the World Trade Centre were born slightly earlier than those outside a two-mile radius (Lederman et al., 2004). Interestingly, risk among women with symptoms of post-traumatic stress disorder (PTSD) or depression was reduced (Engel et al., 2005). Generally, knowing of the disaster had no adverse effects except for a slight shortening of gestation length reported in Dutch women post-9/11 who learned about the disaster through the media (0.7 days, p=0.07) (Smits et al., 2006).

Terrorist attacks. A reduction in birthweight was found for women exposed to terrorist attacks in Colombia in 1998-2003 and during a three-month bombing of Belgrade in Serbia in 1999, but there was no difference in birth length or gestational age at birth (Camacho, 2008, Maric et al., 2010). After the U. S. Embassy bombing in Nairobi in 1998, several women reported, anecdotally, of spontaneous abortions and premature labor (Njenga and Nyamai, 2005).

Environmental and chemical disasters. There was no PTB in association with the Love Canal disaster (identified in 1978) in a population-based cohort (Goldman et al., 1985). The Chernobyl nuclear disaster had no PTB associations directly, but Levi found that anxiety due to Chernobyl, but not the environmental threat itself, was associated with earlier births in a sample of Swedish women (Levi et al., 1989).

Earthquakes. Perhaps because of its severity, the 2008 Chinese earthquake that caused 70,000 deaths was associated with many birth complications, including higher rates of low birth weight, PTB, birth defects, and lower Apgar scores in a large study of survivors (Tan et al., 2009). The 1999 Chi-Chi earthquake in Taiwan, which displaced at least 100,000 people (Chen et al., 2003), was not associated with changes in birth length, in a hospital-based cohort (Chang et al., 2002). Women exposed to the 1994 Northridge earthquake in California gave birth earlier than expected, and the effect was strongest for those exposed in the first trimester (38.06 weeks observed versus 39.29 weeks expected) (Glynn et al., 2001). An increase in PTB was seen in a hospital after two of five Israeli earthquakes, although there was no difference in Apgar scores (Weissman et al., 1989).

Hurricanes and floods. Hurricane Katrina was associated with a fall in very PTB but no change in low birth weight or overall PTB (Hamilton et al., 2009), while a smaller study reported a higher rate of low birth weight and an insignificant increase in PTB after Katrina (Xiong et al., 2008). In flood studies, an increased incidence of PTB and low birth weight was seen among a village in southern Poland in 1997 (Neuberg et al., 1998) and the flooding of the Red River in North Dakota in 1997 (Tong et al., 2011), although there was no increase in small for gestational age in the North Dakota sample.

Storms. An ice storm hit Quebec in 1998, causing widespread power outages to 3 million people for up to 5 weeks (King and Laplante, 2005). Gestational lengths were shorter when women were pregnant during the storm in the first or second trimesters; pre-conceptual or third trimester exposure was not related to PTB (Dancuse et al., 2011). In the 1994 storm-
related sinking of the Swedish ferry Estonia, 501 people died. A subsequent 15% increase in very low birth weight births was reported in the Swedish population (Catalano and Hartig, 2001).

**Overall conclusion of natural disasters.** The authors (Harville et al., 2010) conclude that the empirical literature on pregnant and postpartum women during disaster is limited. Many of the published reports and recommendations are anecdotal or based on the experience and impressions of relief workers (Campbell, 2005) and clinicians (Curballo et al., 2005, Ewing et al., 2008, Pascali-Bonaro, 2002) rather than systematic studies. They fail to examine whole populations and instead largely gather data from women who presented for clinical care or were the most affected. In conclusion, the literature related to disaster-associated adverse pregnancy outcomes, including PTB, is limited and disaster, in and of itself, does not seem to shorten gestation or cause PTB in any consistent fashion.

**Chronic Stress**

In spite of the overall outcome of disaster-related events, there are indeed epidemiological studies that have shown that pregnant women who experience high levels of psychosocial stress before or during pregnancy are at significant risk for PTB regardless of their ethnicity and socioeconomic status (Copper et al., 1996, Dole et al., 2003, International HapMap Consortium, 2005, Neggers et al., 2006, Steer, 2005, Wadhwa et al., 2001). This especially includes women who experienced major and traumatic life events early in pregnancy. Examples given above plus others include Hurricane Katrina (Buekens et al., 2006), the Quebec Ice Storm (Dancause et al., 2011) and the World Trade Center disaster (Lederman et al., 2004) Hedegaard et al., found that major life events during pregnancy were only associated with PTB when they were perceived to be stressful (Hedegaard et al., 1996). Indeed, women who have increased perceptions of stress also have a higher risk of PTB (Austin and Leader, 2000, Orr et al., 2002, Rini et al., 1999). In addition, physically demanding work, prolonged standing, shift and night work, and a high cumulative work fatigue score have been associated with PTB (Luke et al., 1995, Mozurkewich et al., 2000). Perceived racial discrimination can increase the risk of PTB. In Canada, Heaman et al., indicated that spontaneous PTB among Aboriginal women was related to a high level of perceived stress (Heaman et al., 2005). Research suggests that physical and emotional abuse or domestic violence prior to or during pregnancy is associated with PTB (Coker et al., 2004, Covington et al., 2001, Fernandez and Krueger, 1999, Neggers et al., 2004). Distressed states such as depression and anxiety play a role in the onset of PTB (Dayan et al., 2002, Orr et al., 2002). In addition, depression and anxiety can increase a woman’s stress levels. Conversely, high levels of stress can result in the development of depression and anxiety (Stratakis and Chrousos, 1995). As previously mentioned, low socio-economical status is believed to be an important risk factor for PTB (Berkowitz and Papiernik, 1993, Kramer et al., 2000, 2001). Socio-economic disadvantage is associated with unhealthy or risky behaviours, including smoking, alcohol abuse and poor eating habits, perception of increased stress levels, and psychological reactions that influence gestation negatively (Kramer et al., 2001). Indeed, behavioural risk factors, like cigarette smoking, alcohol and drug use, sexually transmitted infections, poor food intake and obesity are all associated with PTB. (Andres and Day, 2000,
Hayatbakhsh et al., 2012, Hendler et al., 2005, Mann et al., 2010, O'Leary et al., 2009, Quesada et al., 2012, Shah and Bracken, 2000).

Christiaens et al., (Christiaens, 2012, Christiaens et al., 2012) assessed chronic, lifelong stressors and related these to spontaneous PTB by designing the ‘Well-being and Pregnancy Questionnaire.’ This is a compilation involving perceived stress (based on the Perceived Stress Scale), common stressors, interpersonal support evaluation list (ISEL, short form), live events check list (CAPS 1), coping (Brief COPE), adverse childhood experiences (ACE), abuse assessment screen (AAS), depression and suicidality (Mini International Neuropsychiatric Interview). Using this questionnaire, both individual and contextual variables that influence the stress response were examined for all subjects. Several checklists designed for this study and validated research instruments were used to measure concepts related to stress and personal resources. Where possible, validated tools that are available in the public domain were used.

Participating subjects were contacted by telephone between three months and one year postpartum where possible for follow-up and administration of the ‘Well-being and Pregnancy Questionnaire.’ To maximise the number of respondents, at least three attempts to contact each participant at different times during days and evenings were made. The questionnaire was administered during the telephone interview, and answers were entered into a secure online database. There were 223 completed telephone questionnaires in the study comprising 148 controls and 75 cases.

Univariate analysis of the socio-demographic and medical variables demonstrated that maternal age, smoking, alcohol use, educational status, and a history of miscarriage were significantly associated with PTB. Physical and emotional abuse as an adult, assessed with the AAS on its own, was not associated with PTB in this study. However, the combined abuse score of childhood and adult abuse was significantly associated with PTB (crude odds ratio (OR) 1.40; 95% CI 1.13-1.74). A significant relationship was found between the computed total stress score and spontaneous PTB after univariate logistic regression, showing a crude OR for the risk of PTB of 1.46, and after dichotomization, a high stress score had an even greater crude OR of 1.86 (95% CI 1.06-3.328). The score for depressive symptoms during pregnancy was significantly associated with PTB (crude OR 1.53; 95% CI 1.01-2.33). A history of major lifetime depression had a fairly high crude OR of 1.70, however this was not significant (95% CI 0.90-3.24).

Of all the separate questionnaire instruments, only the Adverse Childhood Experiences score was significantly associated with spontaneous PTB. Its crude odds ratio when dichotomized into high (≥2 ACEs) versus low ACE, based on median split, was 2.45 (95% CI 1.37-4.38) , which was confirmed with multivariate analysis. When looking more specifically at the relationship between ACE score and spontaneous PTB, it was observed that the proportion of women with PTB gradually increased with a greater number of adverse childhood experiences. Inversely, the percentage of women with a term birth decreased as the number of ACEs increased. When exploring the effect of lifetime abuse – combining childhood and adult abuse scores – it was determined that with each additional increment of 1 on the abuse score scale, the risk of spontaneous PTB increased by 34% (adjusted OR 1.30; 95% CI 1.02-1.65).

It is very likely that ACEs interact with the various sociodemographic and medical risk factors for PTB thereby increasing risk for PTB; unfortunately the sample size in Christiaens’ et al. study was not adequately powered to test for these possible interactions. Adult abuse on
its own was not associated with spontaneous PTB, but when combined with the ACE scores of childhood abuse and neglect and the AAS adult abuse score, a significant relationship between lifetime abuse and PTB was revealed. These data demonstrate that, in this small patient population, that measures of childhood and adult abuse taken together are useful predictors of risk.

Genes and Environment

Cortisol acts on two receptors, the glucocorticoid receptor (GR or NR3C1) and the mineralocorticoid receptor (MR or NR3C2). There is evidence of genetic associations between single nucleotide polymorphisms (SNPs) in genes involved in the HPA axis and adverse mental health outcomes. Several published reports have demonstrated the association between polymorphisms in CRHR1 and depression and suicidality (Boscarino et al., 2012, Bradley et al., 2008, Ishitobi et al., 2012, Liu et al., 2006). In addition, various polymorphisms in the GR gene have been associated with depression and HPA axis regulation (Kumsta et al., 2009, van West et al., 2009). A recent Dutch study found two functional haplotypes in the NR3C2 gene to be associated with perceived chronic stress and increased levels of salivary and plasma cortisol, plasma ACTH and heart rate (van Leeuwen et al., 2011). FK506 binding protein 5 (FKBP5) is part of a receptor complex regulating the sensitivity of the GR (Gillespie et al., 2009). Several SNPs of the FKBP5 gene were found to interact with child abuse and adult post-traumatic stress disorder (Binder et al., 2008, Boscarino et al., 2012, Xie et al., 2010), and were found to increase the risk of depression (Velders et al., 2011).

The serotonin (5HT) pathway is, together with the dopamine pathway, part of the mesocorticolimbic system in the brain and involved in the regulation of stress sensitive mood states. Reuptake and availability of 5HT is controlled by the serotonin transporter (5HTT or SLC6A4) and the importance of this transporter in the regulation of 5HT has long been recognized (Lesch, 2007). A gene-environment interaction study linking childhood abuse, SLC6A4 risk allele and depression found that individuals homozygous for the effect allele and who were abused during childhood had a three-fold increase in their risk of developing major depression (Caspi et al., 2003). The enzymes monoamine oxidase A (MAO-A) and catechol-o-methyltransferase (COMT) control the metabolism of serotonin, dopamine and norepinephrine in the brain. Polymorphisms in the genes encoding for these enzymes have been linked to depression and anxiety (Boscarino et al., 2012, Hettema et al., 2008, Leuchter et al., 2009, Zhang et al., 2010). Christiaens et al., (Christiaens, 2012, Christiaens et al., 2013) studied whether candidate SNPs associated with altered stress response or mental health complications would also be associated with spontaneous PTB.

Seven key genes involved in the stress response and mood regulation were selected for examination using a haplotype approach that provided broad coverage of functional and non-functional SNPs. They code for the corticotropin releasing hormone receptor (CRHR1), the glucocorticoid receptor (NR3C1), the mineralocorticoid receptor (NR3C2), FK506 binding protein 5 (FKBP5), the serotonin transporter (SLC6A4) and the enzymes MAOA and COMT. Tag SNPs for all genes were identified using the TagSNP function on the SNPinfo web server (Xu and Taylor, 2009). All identified tag SNPs were then compared to a database. SNPs that were found to be associated with PTB in this database at \( p \leq 0.05 \) were selected for the
candidate gene study. As a result, 9 tag SNPs in three different genes – NR3C1, NR3C2 and CRHR1 – were studied.

DNA from salivary samples was analyzed from 190 cases and 369 controls. Using a multivariate model that included maternal age, smoking, alcohol use, education, and history of spontaneous abortion as covariates, two SNPs found in the MR, rs1784063 and rs2883929, remained significantly associated with spontaneous PTB after adjustment. In both cases, the effect allele was found to be protective of the risk of spontaneous PTB. For each additional effect allele, the risk of PTB was reduced by an odds of 2.00 and 2.04 for rs1784063 and rs2883929, respectively.

While the GR is present in almost every cell in the body (Lu et al., 2006), the MR occurs mainly in brain areas of the limbic system and the hippocampus (van Leeuwen et al., 2011). The affinity of cortisol for the MR is much higher than for GR, and binding of cortisol to MR is maintained at basal levels while the GR is only activated in response to a stressor. It is thought that the MR mainly regulates basal activity and the stimulation of the HPA axis, while the GR regulates its termination (Joels et al., 2008). Functional polymorphisms in the human NR3C2 gene have been identified (DeRijk et al., 2011, Muhtz et al., 2011, van Leeuwen et al., 2011). These were found to be associated with increased levels of cortisol and psychosocial stress. These candidate gene studies demonstrated that the polymorphisms rs17484063 and rs2883929, both located in the NR3C2 gene coding for the mineralocorticoid receptor are significantly associated with spontaneous PTB in a protective manner. This association is independent of the known PTB risk factors maternal age, smoking status, alcohol use, educational status and history of miscarriage. For each additional effect allele, the risk of PTB was reduced by an odds of 2.00 for rs17484063 and 2.04 for rs2883929. In other words, in women who are heterozygous for the rs17484063 or the rs2883929 effect allele, the risk of spontaneous PTB is halved. If a woman is homozygous for either risk allele, the risk of delivering preterm is further cut in half.

The relationship between genetics and risk for PTB is only 20-40%, and since PTB is a multifactorial disease, the likelihood that the environment can affect gene expression must be examined. Such gene x environment interactions are not uncommon in other complex diseases. For instance, Bogdan et al., observed a significant gene x childhood neglect interaction when studying the effect of a different NR3C2 genotype on amygdala reactivity (Bogdan et al., 2012). And a gene x environment interaction was found when linking childhood abuse, SLC6A4 risk allele and depression (Caspi et al., 2003). Individuals who were homozygous for the effect allele and who were abused during childhood had a three-fold increase in their risk of developing major depression.

Christiaens et al. (Christiaens, 2012, Christiaens et al., 2012, 2013) therefore decided to test whether the presence of protective SNPs for MR in women who also had a low number of ACEs (<2) offered a greater level of protection against PTB risk than either the SNP or low ACEs alone. Indeed, they found that when only women with low ACE scores also had the rs17484063 SNP, they demonstrated an adjusted OR of 0.37 (95% CI 0.16-0.87) for the risk of PTB. Similarly, the analyses of rs2883929 showed comparable results; the adjusted OR of rs2883929 for spontaneous PTB in women with a low ACE score was OR 0.37 (95% CI 0.17-0.81). Combining the genetic effect (SNP) with the environmental effect (low stress) demonstrates more protection against risk for PTB than either alone or added together, although due to the small sample size, this was not a true gene-environment interaction. For women who experienced 0 or 1 adverse childhood events, a preliminary analysis revealed that
each additional risk allele of either rs17484063 or rs2883929 was associated with a 2.7-fold decrease in the risk of spontaneous PTB.

The role of gene-environment interactions in the etiology of PTB is relatively unexplored. Only a few reports examining the role of gene-environment interactions on PTB have been published, and the results have been inconsistent. Some studies found evidence that the presence of an environmental factor, bacterial vaginosis, modifies the genetic association with PTB (Gomez et al., 2010, Macones et al., 2004). There is evidence in the literature that gene x environment interactions also play a role in the development of adverse mental health outcomes, such as post-traumatic stress disorder and depression (Keers and Uher, 2012, Kolassa et al., 2010). Several studies showed an interaction of childhood adverse experience with polymorphisms in predicting depression, post-traumatic stress disorder and alcohol dependence (Bradley et al., 2008, Caspi et al., 2003, 2010, Schellekens et al., 2012).

Animal Studies

A recent study by our laboratories exposed pregnant female Long-Evans rats to transient stress (Yao et al., 2014). Their gestating F1 daughters were either exposed to transient stress again or remained as non-stress controls. Their gestating F2 grand-daughters were again exposed to transient stress or remained as non-stress controls, thus generating two lineages: one of repeated, multigenerational stress and one of in which stress was limited to F0 parental exposure. With each generation, stress in both lineages gradually reduced gestational length, maternal weight gain and maternal behavioural activity, and increased the risk of gestational diabetes. Thus both trans- and multigenerational stress exposure equally modulated gestational length, pregnancy outcomes and offspring health.

Concerning offspring health, delayed offspring development was recognizable as early as postnatal day 7, with the greatest effect in the F3 offspring whose F0 parental generation experienced stress only (Yao et al., 2014). Thus, although context-dependent programming occurs, programming of the germ-line became evident in the F3 generation. In the F3 generation, gestational stress that was imposed on the great-maternal generation seems to have been passed on, via the gametes, to impede development. The F3 generation showed more impairments than any of the other generations in a sensorimotor task that required animals to respond to placement on an inclined ramp with the head facing down (Yao et al., 2014). A failure to quickly turn around and ascend the plane in 7-day old offspring may be indicative of delayed proprioceptive, musculoskeletal, and vestibular development. The striking phenotypic impairments in the F3 generation suggest a genuine transgenerational epigenetic inheritance whereby the epigenetic modifications have been passed via the gametes that have escaped reprogramming (Migicovsky and Kovalchuk, 2011, Zucchi et al., 2012).

The phenotypic findings in behaviour and physiology were supported by molecular changes involving epigenetic regulation of gene expression in the brain and uterus. Our study indicated that the multigenerational stress altered microRNA (miRNA) expression patterns in brain and uterus of F2 mothers, including the miR-200 family (Yao et al., 2014). In particular, stress led to upregulation of miR-200b and downregulation of miR-429. In the uterus, both miR-200b and miR-429 were suggested to modulate gestational length through interaction with their gene targets Stat5b, Zeb1 and Zeb2 (Renthal et al., 2010). The data indicated that
when upregulated, miR-200b may suppress Stat5b, Zeb1 and Zeb2 mRNA levels in the lineage that exposed each generation of pregnant dams to prenatal stress (i.e., the F2 offspring from stressed F0 and F1 generations). Furthermore, stress also increased miR-181a expression in the placenta (Yao et al., 2014) miR-181a has been associated with PTB in humans and may serve as a marker of shortened gestation (Mayor-Lynn et al., 2011). These findings suggest that the mechanisms involved in the timing of parturition and associated behavioural and physiological signatures may be programmed through the maternal lineage. The identification of epigenetic signatures of PTB in clinically accessible tissues, such as placenta, offers the potential for predictive and preventive studies related to poor pregnancy outcomes.

Transgenerational studies. A healthy pregnancy starts long before conception. Cumulative effects of stress throughout the lifetime and even in ancestors seem to be particularly important for PTB risk (Rich-Edwards and Grizzard, 2005). Such cumulative effects may include increased sensitivity to stress due to programming by stress in the ancestors. Interestingly, elevated PTB risk was noted to propagate through generations (Porter et al., 1997), suggesting that factors determining PTB risk may be passed on to the offspring through the maternal lineage. Our data suggest that indeed that exposure of a mother, grandmother or great-grandmother to gestational stress may increase the risk of PTB. While high levels of psychological stress have been positively correlated with PTB in humans (Hedegaard et al., 1993, Hobel, 2004), the causal link may involve the endocrine regulation and cytokine milieu for maintenance of pregnancy and timing of delivery (Arck, 2001, Coussons-Read et al., 2012, Miller and Riegle, 1985). Protective mechanisms, such as a down-regulation of the HPA axis activity during pregnancy, add a level of endocrine complexity to the study of stress influences on pregnancy outcomes (Glynn et al., 2001). The timing and severity of the stressor are critical in that stress in early pregnancy has greater effects on gestation length than stress experienced in the last trimester (Glynn et al., 2001).

Mechanisms of prenatal stress to modulate gestational length likely include the modulation of pro-inflammatory pathways leading to PTB (Arck, 2001). Furthermore, stress may alter relevant neuropeptide and hormone levels, including prolactin, progesterone, and oxytocin, which are involved in pregnancy maintenance and the timing of parturition (Miller and Riegle, 1985). Moreover, fetal HPA axis stimulation may induce prostaglandin production by fetal membranes and decidua and result in uterine activation (Christiaens et al., 2008). Stress may also stimulate cytokines, which regulate the activity of placental 11-beta-hydroxysteroid dehydrogenase (type 2) (Kossintseva et al., 2006) to elevate PTB risk. Based on these endocrine regulations by early experience it was proposed that PTB risk may have roots in childhood (Rich-Edwards and Grizzard, 2005).

Possible mechanisms of transgenerational transmission of PTB risk may be linked to a stress-associated epigenotype involving microRNAs (miRNAs) that are replicated in subsequent generations. The rapid response of epigenetic components to maternal stress may represent the intersection of genetic and environmental factors determining PTB risk. Thus, the characterization of epigenetic regulators of genes or single nucleotide polymorphisms and their environmental regulation may provide exciting new predictive signatures and therapeutic interventions for PTB and associated health outcomes.
Mediators

Evidence suggests that adverse childhood experiences can lead to hyperreactivity of the HPA and SAM axis in response to stress in adulthood (Heim et al., 2000). This effect is even stronger in women with symptoms of depression. It is believed that ACEs can induce persistent changes in the systems involved in the stress response leading to negative health outcomes such as depression (Heim et al., 2001). This is in complete agreement with the concept of allostatic load as described earlier in this chapter. It is biologically very plausible that lifetime experience, including some disaster experiences, chronic stress, and adverse childhood experiences, can increase the risk of PTB via the inflammatory, neuroendocrine and epigenetic pathways.

Uterine-Placental Endocrine-Immune Mechanisms

Parturition at term or preterm requires a transformation of the uterus of pregnancy to the uterus of delivery as evidenced by changes in the expression and levels of uterine transformation proteins (UTFs). Typically, the prostaglandin F (PGF$_{2\alpha}$) receptor (PTGFR), oxytocin receptor, inducible cyclooxygenase-2 and gap junction protein, connexin-43, are UTFs used as proxies to monitor changes in dozens of ‘transforming’ proteins. The process of transformation requires three integrated components, positive feedback, synergy and amplification. Positive feedback involves the actions of key mediators, such as IL-1β, PGF$_{2\alpha}$ and CRH whereby they each stimulate expression of several cytokines, chemokines, CRH, PGs, receptors including PTGFR, and, in the case of IL-1β, its own receptor and accessory protein (Erickson et al., 2001, Ishiguro et al., 2014, You et al., 2014). In the fetal membranes and placenta, maternal cortisol can drive both CRH and PG synthesis (Challis et al., 1999, Erickson et al., 2001, Jones et al., 1989, Karalis et al., 1996, Li et al., 2014, Smith, 1998). Hence elevated levels of maternal cortisol could potentially initiate positive feedback in the uterine tissues.

Synergy is where the action of two or more mediators is more than additive of their actions alone in stimulating the events in positive feedback. An excellent example of this is when PGF$_{2\alpha}$ and IL-1β together stimulate the mRNA expression of IL-6 in human uterine smooth muscle cells several hundred-fold, which is multiples of their independent actions (Leimert et al., 2014). Amplification involves the recruitment of peripheral leukocytes to the uterine tissues whereby the release of their mediators amplifies the action of transformation. Amplification is achieved when mediators such as CRH, PGF$_{2\alpha}$ or IL-1β stimulate the release of chemoattractants from uterine tissues. Recent evidence demonstrates that CRH releases a myometrial chemoattractant (You et al., 2014).

A further consequence of upregulating uterine transformation might be that cytokines produced on the maternal side of the placenta inhibit the enzymatic action of 11BHSD2 (Kossintseva et al., 2006). This would allow more maternal cortisol to cross the placenta where the above positive feedback actions could occur on the fetal side of the placenta and in fetal membranes, leading to increases in fetal PGs, cytokines and CRH.
Neuroendocrine Mechanisms

During pregnancy either progesterone produced by the corpus luteum or by the placenta in species with long gestations can be converted via 5α-reductase, found in both the placenta and in the maternal and fetal brain cortex, into allopregnanolone, a neurosteroid that is found in both the maternal and fetal circulation. Allopregnanolone does not interact with the traditional nuclear progesterone receptors (PR) A or B, but rather with the GABA_A membrane receptor, whereby it promotes chloride ion flux. It has a significant role in influencing the fetal central nervous system in late pregnancy in part by promoting neuronal growth, neuroprotection or normal cognition and behaviour, and by contributing to the sleep-like behavioural traits typical of the fetus (Hirst et al., 2009). One of the most important roles for allopregnanolone is to suppress the release of neurohypophyseal oxytocin, a uterine contractile agonist (Brunton et al., 2014).

A number of stressors in pregnancy, such as restraint stress (Frye and Walf, 2004), reduce brain allopregnanolone levels, whereas others such as hypoxic ischemia, increase allopregnanolone production (Kelleher et al., 2011). Being born preterm separates the fetus from its placental source of progesterone and puts it at considerable risk, especially to hypoxic stress. This vulnerability may be even greater in the IUGR fetus, which is likely to already have compromised growth trajectories.

Epigenetic Mechanisms

As discussed in the sections above, environmental conditions, lifestyle and stress may be linked to altered inflammatory status, health and disease, and even elevated PTB risk. A central mechanism how such dynamic gene-environment interactions are made possible is by epigenetic regulation that mediates rapid adaptation of gene expression patterns to diverse environments and experiences. Epigenetics is the study of heritable changes in gene activity without altering the DNA sequence (Migicovsky and Kovalchuk, 2011). The major epigenetic events include DNA cytosine methylation, histone modifications and post-transcriptional control through microRNA (miRNA) expression. The vital importance of epigenetic regulation in regulating gene expression is illustrated during development and maturation (Iyengar et al., 2014, Kolb et al., 2012). While each cell in a given organism shares the same genome, epigenetic regulation of gene expression allows cells, tissues, and organs to differ because certain sets of genes are "turned on" or expressed, while other sets are "turned off" or inhibited.

Considering the striking inter-generational programming of ancestral stress on PTB risk and other health complications, it is important to consider the potentially central role of inheritance of epigenetic marks. Epigenetic mechanisms may mediate a gradually altering physiological response to recurrent stress across various generations of individuals. The formation of an epigenetic memory to a single or recurrent adverse event within a family history may assist in adjusting physiological and/or behavioural patterns to a stressful environment. Epigenetic memory refers to transgenerationally stable, yet dynamic re-programming of the germline epigenome that transfers information across generations (Migicovsky and Kovalchuk, 2011, Skinner, 2008). Such heritable epigenetic changes facilitate rapid adaptation to adverse environmental conditions, but may also result in a
mismatch of physiological profiles to later-life challenges, thus enhancing disease risk. Importantly, tissue-specific miRNA signatures can be found in blood plasma (Laterza et al., 2009), emphasizing the possibility to determine epigenetic signatures of prognostic significance for disease. Many common health conditions share a suspected etiology that includes both the influence of adverse perinatal origins as well as a transcriptomic component, suggesting that epigenetic regulation of gene expression may represent a central unifying feature in individual complex disease etiology (Petronis, 2010).

Transgenerational programming of PTB risk may be linked to a stress-associated epigenotype involving miRNAs that are transferred to subsequent generations. miRNAs are reasonable candidates for such a role since they are differentially regulated by progesterone during myometrial quiescence and initiation of parturition (Renthal et al., 2010, Williams et al., 2012). Including the downregulated miR-200b, the miR-200 family may exert peripheral effects to control uterine quiescence and contractility during pregnancy and labour (Renthal et al., 2010). Interestingly, members of the miR-200 family are expressed in term labour in mice and humans and upregulated in mouse models of preterm labour (Renthal et al., 2010). Members of the miR-200 family may specifically interact with the endocrine cascade involved in pregnancy maintenance and termination, including progesterone and oxytocin (Renthal et al., 2010).

Target genes of the miR-200 family include three particular genes, Stat5b, Zeb1 and Zeb2, involved in pregnancy maintenance. In our study by Yao et al., (Yao et al., 2014), all three were downregulated by multigenerational stress in the uterus of dams of the F1 generation. The downregulation of Zeb2 expression was still present in the F2 generation (Yao et al., 2014), and thus may be linked to the observed reduction in gestational length among F1 and F2 dams (Yao et al., 2014). A decrease in Stat5b expression was also linked to reduced progesterone activity and the initiation of labor, particularly PTB (Williams et al., 2012). Furthermore, ZEB1 serves as a transcription factor to inhibit the miR-200 family, thus enhancing Stat5b expression) (Williams et al., 2012). As the myometrium transitions to term or preterm labor, reduced progesterone activity decreases ZEB1 and ZEB2 levels via a feed-forward mechanism (Renthal et al., 2010, Williams et al., 2012), thus regulating the timing of parturition. The upregulation of uterine miR-200b may be causative for the suppression of Stat5b and ZEB1 and ZEB2. Differential expression of these components across generations coincides with shortened gestational length and indicates a causal or at least predictive signature of PTB.

Perhaps the most common, and the most widely studied, epigenetic modification is DNA methylation. DNA methylation occurs primarily at CpG sites of the DNA strand. Cytosines in CpG dinucleotides can be methylated, i.e., one or more methyl groups are appended to form 5-methylcytosine by enzymes called DNA methyltransferases. In mammals, methylating the cytosine within a gene can turn the gene off and suppress the expression of this gene. By contrast, hypomethylation of a CpG site is commonly associated with over-expression of the respective gene. Notably, in the human genome ~70-80% of cytosines in CpG dinucleotides are methylated (Ziller et al., 2013). Most cell types, except germ cells and pre-implantation embryos, maintain relatively stable DNA methylation patterns and only a fraction of CpG sites will alter methylation status as part of a coordinated regulatory program (Ziller et al., 2013). Consequently, DNA methylation patterns are highly tissue-specific. Importantly, a large body of evidence suggests that stress is a potent influence to alter DNA methylation patterns.
In rat mothers exposed to gestational stress, the buffering action of placental HSD11B2 may be reduced by downregulation of this enzyme (Mairesse et al., 2007, Sarkar et al., 2001). For example, restraint stress during gestational days 11–20 in rats was shown to decrease placental 11β-HSD2 enzymatic activity and also decrease mRNA levels of this gene (Mairesse et al., 2007). Furthermore, a study by Jensen Peña and colleagues (2012) provided insights into underlying epigenetic regulation associated with altered HSD11B2 function. Here, prenatal stress increased placental mRNA levels of the DNA methyltransferase, DNMT3a, and increased DNA methylation at specific CpG sites within the HSD11B2 gene promoter, thus reducing gene expression activity at this site. Based on a comparative analysis of placenta and fetal brain, the authors also proposed that placental DNA methylation status has clinical predictive value for brain (Jensen Pena et al., 2012).

Altered DNA methylation has also been suggested to be partially responsible for the significant developmental consequences of prenatal stress in offspring brain and behaviour. For example, prenatal stress in rats alters the expression of DNA methyltransferases (Dnmt) 1 and 3a in the amygdala and hippocampus, and increases DNA methylation of exon IV of the gene for brain-derived neurotrophic factor (BDNF), a vital neurotrophic factor for brain development and axonal guidance (Boersma et al., 2014). These findings may in part explain altered affective behaviours, such as anxiety, in prenatally stressed rats, but also developmental delays associated with the exposure to elevated glucocorticoid levels in utero. These findings are in line with a recent study of pregnant women exposed to extreme maternal psychosocial stress in the Democratic Republic of Congo (Mulligan et al., 2012). In this study, exposure to severe stress in the mother was positively associated with low newborn birth weight and elevated newborn methylation in the promoter of the glucocorticoid receptor NR3C1, thus reducing the expression of glucocorticoid receptors and potentially impairing the ability of negative feedback regulation of the newborn’s stress response (Mulligan et al., 2012). The authors suggest that increased methylation of the NR3C1 gene and the subsequently reduced GR density in the brain, such as the hippocampus, of affected newborns may ultimately reduce the range of stress adaptation responses, thus increasing their risk for adult-onset diseases (Mulligan et al., 2012). These findings support the increasingly prominent notion that many complex adult onset diseases, including the risk of preterm birth, share a multifactorial etiology.

Integration

Two-hit hypothesis. To explain the etiology of many diseases that involve a complex pathogenesis, the “multiple-hit” hypothesis has been proposed. This model has been originally developed by Nordling (1953) and Knudsen (1971) to explain the pathogenesis of cancer (Knudson, 1971, Nordling, 1953). The model proposes that genetic and environmental factors disrupt early cell and/or organ development and generate a long-term vulnerability to a “second hit” that then leads to the onset of disease symptoms. This model is now widely accepted when neither genetic nor environmental factors can be clearly identified to cause a disease. In the case of PTB, genetic factors or adverse environmental stimuli in the womb, such as maternal stress, may set the stage for endocrine and inflammatory dysregulation without any significant symptoms. A second event in adolescence or early adulthood, however, may then trigger or worsen the pathological condition and transform a previously
“silent” condition into an overt health problem. In the case of PTB, such a "second hit” may be a major life event or an episode of stress.

**Conclusion and Future Work**

Evidence is accumulating that links stress, especially chronic and transgenerational stress, with PTB. The human data are less convincing than the animal data, but that derives mostly from the difficulty of systematically studying the problem in natural experiments that rely upon lifetime experience or natural disaster to introduce the insult that results in an adverse pregnancy outcome. The animal models are improving and through them investigators will be able to ascertain mechanisms and pathway points of convergence that will both identify markers to study in humans and potential therapeutic targets. An immediately achievable goal, even with the human data already available, should be to develop tools based upon lifetime experiences for assessing populations of vulnerable women for PTB risk as early as possible in pregnancy. This would enable closer surveillance of their pregnancies. Simple, low-cost tools capable of identifying women at the greatest risk would provide tremendous advances in improving women’s pregnancy and newborn health and possibly ameliorate adverse transgenerational health outcomes.

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