

Role of foetal programming and epigenetic mechanisms in the pathogenesis of arterial hypertension

Emrush Rexhaj, Stefano F. Rimoldi, Roman Brenner, Agim Pireva, David Cerny, Robert Von Arx, Claudio Sartori, Urs Scherrer, Yves Allemann

Department of Cardiology, Inselspital, Bern, Switzerland

Summary

Essential hypertension remains poorly understood, despite the enormous efforts of research, which brought understanding of certain physiopathological and treatment mechanisms. New insights have emerged from recent data showing that environmental insults during (*in vitro*) fertilisation, foetal, perinatal and childhood periods may lead to vascular dysfunction and hypertension later in life (the foetal programming of adult disease hypothesis, also called the “Barker hypothesis”). In line with this hypothesis, we recently reported that apparently young healthy children born after a pregnancy complicated by preeclampsia or born after *in vitro* fertilisation display a marked generalised vascular dysfunction, which may predispose them to premature cardiovascular morbidity and mortality. New data from animal studies strongly suggest that epigenetic alterations are important underpinning mechanisms involved in these vascular abnormalities. Preliminary animal data suggest that pharmacological interventions targeted at these epigenetic modifications may avoid premature cardiovascular morbidity and mortality.

Key words: hypertension; vascular dysfunction; in vitro fertilisation; preeclampsia; foetal programming of adult disease hypothesis; Barker hypothesis; epigenetics

Introduction

Hypertension affects >1 billion people worldwide [1]. In Switzerland, >50% people older than 60 years are hypertensive [2]. Hypertension is the most prevalent risk factor for cardiovascular mortality worldwide [3]. Whereas in roughly 10% of hypertensive patients an underlying cause can be identified, in the remaining 90% the origin of high blood pressure remains largely

unknown and is labelled as “essential” hypertension. Epidemiological and observational studies have shown that hypertension occurs more frequently in some ethnicities or families, suggesting a genetic

mechanism to the disease. Indeed, heritability of hypertension is estimated to be between 30% and 40% [1, 4]. However, so far genetic variants, individually and collectively, explain only a small fraction of the phenotypic variation and disease risk [1]. What are the possible alternative explanations for this so called “missing heritability”? Several hypotheses have been proposed, including overestimates of heritability, unexplored regions of the genome, untested classes of genetic variants, the action of many rare genetic variants, and gene interactions [1]. Phenotypic variation can also be regulated independent of changes in DNA sequence, thereby escaping detection with classic genetic approaches. These heritable epigenetic changes could explain some of the missing heritability in hypertension.

Furthermore, epidemiological data in humans during the last 20 years suggest that environmental factors acting during the foetal and perinatal period induce alterations that predispose to metabolic and/or cardiovascular disease later in life. These observations have led to the so-called “foetal programming of adult disease hypothesis” or “Barker hypothesis” [5]. For example, studies in animals showed that maternal undernutrition results in increased systolic and diastolic blood pressure in the offspring [6]. In humans, maternal malnutrition during early gestation (The Dutch famine studies) increases the prevalence of coronary heart diseases in the offspring [7]. In line with the foetal programming of adult disease hypothesis, we recently reported that apparently healthy young children born after a pregnancy complicated by preeclampsia or born after *in vitro* fertilisation display a marked generalised vascular dysfunction which may predispose them to premature cardiovascular morbidity and

Funding / potential competing interests:

No financial support and no other potential conflict of interest relevant to this article was reported.

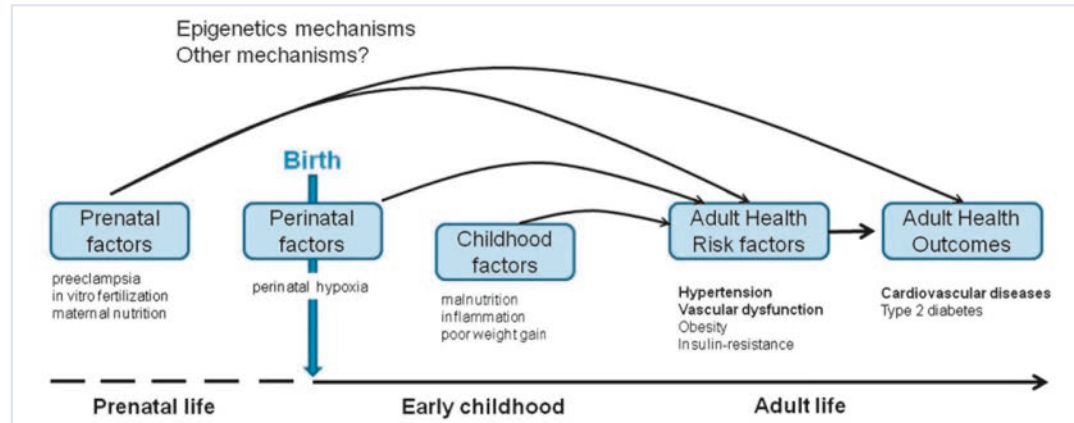
Correspondence:

Professor Yves Allemann, MD
Swiss Cardiovascular Center Bern,
Cardiology University Hospital, Inselspital
CH-3010 Bern
Switzerland
yves.allemann[at]insel.ch

mortality [8, 9] (fig. 1). In line with this concept, the Helsinki birth cohort demonstrated that adult offspring of a mother with preeclampsia are at increased risk of stroke [10]. We speculated that epigenetic mechanisms underpin the generalised vascular dysfunction observed in offspring of women with preeclampsia and *in vitro* fertilised children. Our hypothesis was based

on the observation that there is high epigenetic activity during embryonic and late foetal development. Insults occurring during this period may affect the normal epigenetic activity, which, in turn, may alter gene expression and function. To test our hypothesis we have generated mouse models of preeclampsia and *in vitro* fertilisation (see below).

Figure 1
Schematic representation of the effects of environmental factors occurring during early development on cardiovascular diseases later in life. The main putative prenatal, perinatal and childhood factors that may lead to epigenetic modifications and in turn influence adult health outcomes are represented.

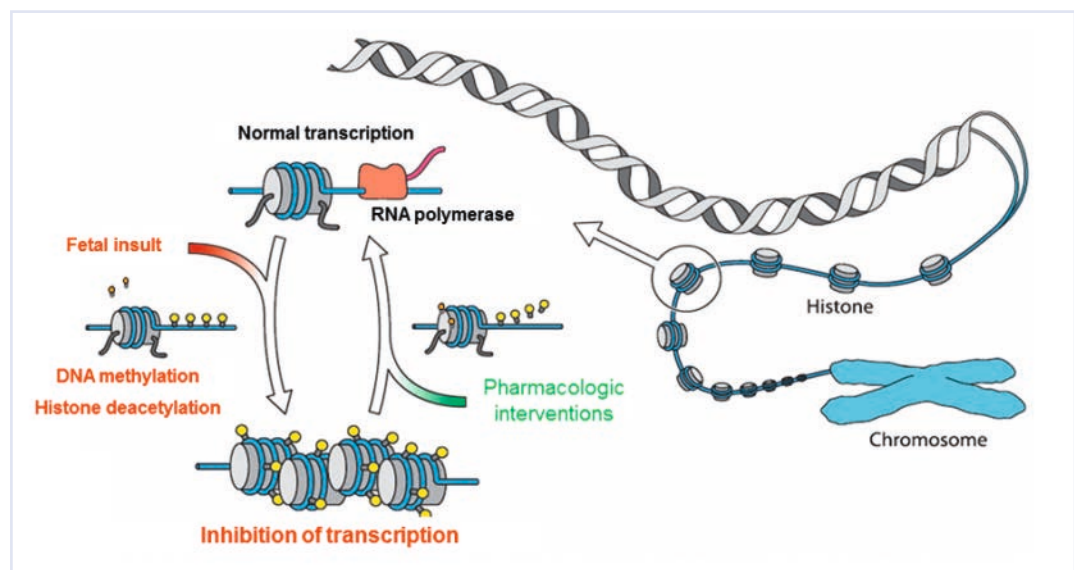


What are epigenetic mechanisms?

The term “epigenetics” refers to important chromatin-based mechanisms that regulate gene expression without affecting the DNA sequence *per se*. Important epigenetic modifications take place during embryonic, foetal and early postnatal development, and are indispensable for the adequate regulation of gene expression, but are strongly influenced by environmental

interactions. There is evidence that epigenetic modifications are heritable throughout generations. The best studied epigenetic mechanisms involve DNA methylation and histone modifications (acetylation and methylation) (fig. 2). Other mechanisms, such as microRNAs, have been also described [1].

Figure 2
Foetal/perinatal insults may induce epigenetic alterations such as DNA methylation and histone deacetylation that will modify gene expression. DNA methylation occurs at CpG dinucleotides and is responsible for inhibition of transcription. Hyperacetylation of the histones correlates with transcriptional activation, whereas their deacetylation is associated with transcriptional inactivation. Epigenetic alterations can be reversed by pharmacological agents, such as histone deacetylase inhibitors.



Preeclampsia

Preeclampsia is characterised by hypertension, proteinuria and oedema, and complicates up to 10% of pregnancies in Western countries. As stated above, we found that young apparently healthy adolescents born after a preeclamptic pregnancy display marked vascular dysfunction in the pulmonary and systemic circulation, as evidenced by a 30% higher pulmonary artery pressure and a 30% smaller flow-mediated dilation (FMD) of the brachial artery compared with control subjects [8]. Vascular dysfunction in the offspring of mothers with preeclampsia could be related to preeclampsia *per se* or to a genetic abnormality that predisposes the mother to preeclampsia and the offspring to vascular dysfunction. To distinguish between these two possibilities, we assessed vascular function in siblings of offspring of mothers with preeclampsia who were born after a normal pregnancy. These siblings had normal pulmonary artery pressure and FMD [8], indicating that preeclampsia *per se* causes vascular dysfunction in the offspring. What are the mechanisms that occur during preeclampsia and cause a persistent defect in the circulation of the offspring? During preeclampsia in humans, oxidative stress, a potent inducer of epigenetic alterations [11], is increased in the circulation of the mother [12]. We speculated that oxygen species cross the placental barrier [13] and cause epigenetic alterations of genes involved in the regulation of the endothelial function of the foetal circulation, and that these modifications would persist during the whole life. To test this hypothesis, we used a mouse model of increased oxidative stress during gestation [14], the restrictive diet pregnancy (RDP) model. Pulmonary vascular function *in vitro*, and pulmonary artery pressure and right ventricular responses to hypoxia *in vivo* were assessed in offspring of RDP and in control mice [15]. To test for epigenetic modifications we assessed pulmonary DNA methylation. Endothelium-dependent pulmonary artery vasodilation *in vitro* was impaired, and hypoxia-induced pulmonary hypertension and right ventricular hypertrophy *in vivo* were exaggerated in offspring of RDP compared with control mice. This pulmonary vascular dysfunction was associated with altered lung DNA methylation. Since epigenetic changes may be reversed by histone deacetylase inhibitors [16] such as butyrate (fig. 2), in a next step, we tested the effects of butyrate administration to offspring of RDP on pulmonary DNA methylation and pulmonary vascular function and found that the administration of butyrate normalized pulmonary DNA methylation and vascular function.

Since oxidative stress is increased in the mother during RDP and possibly triggers epigenetic changes and vascular dysfunction in the offspring, we examined

the potential preventive effects on epigenetic and vascular modifications in the offspring by administering the antioxidant tempol to the mother during RDP. Indeed, administration of tempol to the mother prevented pulmonary DNA demethylation and vascular dysfunction in the offspring, confirming that increased oxidative stress triggers pulmonary vascular dysfunction in the offspring by an epigenetic mechanism. A similar mechanism may be involved in the foetal programming of vascular dysfunction induced by preeclampsia in humans.

Assisted reproductive technology

Assisted reproductive technology (ART) has allowed millions of infertile couples worldwide to have children and now make up 1% to 4% of the births in developed countries. ART involves the manipulation of embryos at early stages of their development when they may be particularly vulnerable to environmental influences that may induce epigenetic alterations, which will persist for the whole lifespan. Therefore, the safety of ART for long-term health is of utmost importance. To assess the cardiovascular function of young and apparently healthy offspring born from ART we measured their systemic [FMD, pulse-wave velocity (PWV) and intima-media thickness (IMT)] and pulmonary (pulmonary artery pressure by Doppler echocardiography) vascular function and compared it with control children [9]. The results show that healthy children conceived by ART display a generalised vascular dysfunction, as evidenced by a 25% smaller FMD, a 16% faster PWV, a 10% greater IMT and a 30% higher pulmonary artery pressure compared with control children. There is good evidence that the vascular dysfunction observed is related to ART procedures *per se*, because vascular function in parents, in children conceived after ovarian hyperstimulation, or in naturally conceived siblings of ART children was normal [9].

Increased blood pressure has been reported in animal models of *in vitro* fertilisation and in children conceived by ART [17, 18]. To further understand the mechanisms involved in ART-induced vascular dysfunction and hypertension, we developed a mouse model of ART. Preliminary data show that mice conceived by *in vitro* fertilisation, like human offspring of ART, display vascular dysfunction and hypertension compared with control mice. Moreover, there is evidence that epigenetic mechanisms involving demethylation of genes responsible for vascular function contribute to the described vasculopathy. Further studies are needed to confirm these data both in mice and in humans. Once this has been done, the next goal will be to find out how ART-induced vascular dysfunction can be prevented.

Conclusion

Essential hypertension remains poorly understood, despite the enormous efforts of research, which brought understanding of certain physiopathological and treatment mechanisms. New insights have emerged from recent data showing that environmental insults during (*in vitro*) fertilisation, foetal, perinatal and childhood periods may lead to vascular dysfunction and hypertension later in life (the foetal programming of adult disease hypothesis) (fig. 1). It has become more and more evident that epigenetic alterations are important underpinning mechanisms involved in these vascular abnormalities. Preliminary animal data from our group suggest that pharmacological interventions targeted at these epigenetic mechanisms avoid premature cardiovascular morbidity and mortality. This should be very stimulating news for future research in humans!

References

- Cowley AW, Jr., Nadeau JH, Baccarelli A, Berecek K, Fornage M, Gibbons GH, et al. Report of the national heart, lung, and blood institute working group on epigenetics and hypertension. *Hypertension*. 2012; 59:899–905.
- Nedelchev K, Arnold M, Baumgartner R, Devuyt G, Erne P, Hayoz D, et al. Vascular risk factors in the swiss population. *J Neurol*. 2005; 252:1210–6.
- Stephan S, Lim ea. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: A systematic analysis for the global burden of disease study 2010. *Lancet*. 2012;380:2224–60.
- Allemann Y, Hutter D, Aeschbacher BC, Fuhrer J, Delacretaz E, Weidmann P. Increased central body fat deposition precedes a significant rise in resting blood pressure in male offspring of essential hypertensive parents: A 5 year follow-up study. *J Hypertens*. 2001;19:2143–8.
- Barker DJ. The foetal and infant origins of disease. *Eur J Clin Invest*. 1995;25:457–63.
- Van Abeelen AF, Veenendaal MV, Painter RC, De Rooij SR, Thangaratnam S, Van Der Post JA, et al. The foetal origins of hypertension: A systematic review and meta-analysis of the evidence from animal experiments of maternal undernutrition. *J Hypertens*. 2012;30:2255–67.
- Roseboom TJ, van der Meulen JH, Osmond C, Barker DJ, Ravelli AC, Schroeder-Tanka JM, et al. Coronary heart disease after prenatal exposure to the dutch famine, 1944–45. *Heart*. 2000;84:595–8.
- Jayet PY, Rimoldi SF, Stuber T, Salmon CS, Hutter D, Rexhaj E, et al. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. *Circulation*. 2010;122:488–94.
- Scherrer U, Rimoldi SF, Rexhaj E, Stuber T, Duplain H, Garcin S, et al. Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. *Circulation*. 2012;125:1890–6.
- Kajantie E, Eriksson JG, Osmond C, Thornburg K, Barker DJ. Preeclampsia is associated with increased risk of stroke in the adult offspring: The helsinki birth cohort study. *Stroke; a journal of cerebral circulation*. 2009;40:1176–80.
- Weitzman SA, Turk PW, Milkowski DH, Kozlowski K. Free radical adducts induce alterations in DNA cytosine methylation. *Proc Natl Acad Sci U S A*. 1994;91:1261–4.
- Gupta S, Aziz N, Sekhon L, Agarwal R, Mansour G, Li J, Agarwal A. Lipid peroxidation and antioxidant status in preeclampsia: A systematic review. *Obstetrical & gynecological survey*. 2009;64:750–9.
- Franco Mdo C, Dantas AP, Akamine EH, Kawamoto EM, Fortes ZB, Scavone C, et al. Enhanced oxidative stress as a potential mechanism underlying the programming of hypertension in utero. *J Cardiovasc Pharmacol*. 2002;40:501–9.
- Vieira-Filho LD, Lara LS, Silva PA, Luzardo R, Einicker-Lamas M, Cardoso HD, et al. Placental oxidative stress in malnourished rats and changes in kidney proximal tubule sodium ATPases in offspring. *Clin Exp Pharmacol Physiol*. 2009;36:1157–63.
- Rexhaj E, Bloch J, Jayet PY, Rimoldi SF, Dessen P, Mathieu C, et al. Foetal programming of pulmonary vascular dysfunction in mice: Role of epigenetic mechanisms. *Am J Physiol Heart Circ Physiol*. 2011;301: H247–52.
- Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004;7:847–54.
- Chen M, Norman RJ, Heilbronn LK. Does *in vitro* fertilisation increase type 2 diabetes and cardiovascular risk? *Curr Diabetes Rev*. 2011;7: 426–32.
- Watkins AJ, Platt D, Papenbrock T, Wilkins A, Eckert JJ, Kwong WY, et al. Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure. *Proc Natl Acad Sci U.S.A*. 2007;104:5449–54.