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Elabela and Apelin actions in healthy and pathological pregnancies

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ABSTRACT

Pregnancy is a dynamic and precisely organized process during which one or more baby develops. Embryonic development relies on the formation of the placenta, allowing nutrient and oxygen exchange between the mother and the fetus. Dysfunction of placental formation lead to pregnancy disorders such as preeclampsia (PE) with serious deleterious consequences for fetal and maternal health. Identifying factors involved in fetoplacental homeostasis could inform better diagnostic and therapeutic strategies for these pathological pregnancies. Here, we summarize actions of elabela, apelin and their common receptor APJ in the fetoplacental unit. Studies indicate that elabela is crucial for embryo cardiovascular system formation and early placental development, while apelin acts in mid/late gestation to modulate fetal angiogenesis and energy homeostasis. Most of these findings, drawn from animal models, indicate a key role of elabela/apelin-APJ system in the fetoplacental unit. This review also provides an overview of clinical studies investigating elabela/apelin-APJ system in pathological complicated pregnancies such as PE and gestational diabetes mellitus (GDM). While elabela-deficient mice display all the features of PE, current clinical studies show no difference in circulating elabela levels between PE and control patients which does not support a role in PE development. Conversely, apelin levels are increased during PE, but the use of apelin as an early PE marker remains to be fully investigated.

1. Introduction

Pregnancy is a dynamic and precisely organized process during which one or more baby develops. The formation of an embryo and later a fetus relies on coordinated spatial and temporal changes in cell division, growth and differentiation. Embryonic development and successful pregnancy outcome also relies on the formation of the placenta, which allows nutrient and oxygen exchange between the mother and the fetus. The placenta functions as a fetomaternal organ with two components, the fetal placenta which develops from the blastocyst to become trophoblast cells, and the maternal placenta which develops from the maternal uterine tissue. In addition to its role in fetal nutrition, the placenta can also release hormones and growth factors that are critical for fetal development and maternal adaptation to pregnancy. Failure of placental formation or functions may lead to miscarriage or pregnancy disorders such as preeclampsia (PE), intrauterine growth restriction (IUGR) and pre-term birth which can all have serious short and long-term consequences for fetal and maternal health. Identifying factors involved in fetoplacental homeostasis could inform better diagnostic and therapeutic strategies for these pathological pregnancies. This review first provides an overview of actions of elabela, apelin and their common receptor APJ in the fetoplacental unit during physiological pregnancy. We then summarize findings on elabela/apelin-APJ systems in complicated pregnancies associated with PE, IUGR or gestational diabetes mellitus (GDM) to evaluate their potential diagnostic and therapeutic roles. Where possible, we draw upon findings from human clinical studies and animal models. While differences exist between species in fetoplacental physiology (e.g. placental structure, duration of gestation, rate of fetal growth), the use of different models still provides insight into the role of these systems and their potential use in therapeutics and diagnostics of pregnancy disorders.

2. Apelin, Elabela and their common receptor APJ: discovery, structure and regulation

The apelin receptor (also known as APJ or APLNR) is a class A G-protein-coupled receptor (GPCR) that was discovered in 1993 based on its sequence homology with the angiotensin II type 1 (AT1) receptor

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[1]. However, APJ does not bind angiotensin II [1]. Its first endogenous ligand was discovered few years later as the hormone apelin [2]. The human apelin gene (*APLN*) encodes a 77-amino acid precursor (pre-proapelin) which is then cleaved from its C-terminus to produce several apelin isoforms of different lengths such as apelin-36, apelin-17, apelin-13 and post-translationally modified pyroglutamate-apelin-13 ((Pyr^1) apelin-13). All isoforms are capable of signaling via APJ [3]. Depending on the cell type, APJ activation can be coupled to different G protein subtypes and trigger several signaling pathways such as the activation of AMPK, PI3K/Akt or MAPK signal transduction pathways and the inhibition of cAMP production [3]. Bovine, human, rat and mouse preproapelin precursors have 76–95% homology, and human, rat and mouse APJ display 89–91% homology [4], indicating that this system is well conserved across a wide range of mammals.

Apelin and APJ are expressed in various tissues of both embryo and adult including heart, vasculature (particularly in endothelial cells), lung, adipose tissue, gastrointestinal tract, several regions of the central nervous system, mammary gland and placenta [5–9]. Because apelin expression pattern generally follows that reported for its receptor APJ, apelin is thought to act in a paracrine/autocrine fashion. Nevertheless, apelin is found in the blood circulation and thus exert endocrine functions. To date, it is not clear which tissues contribute the most to circulating apelin level. In human, apelin-13, (Pyr^1)apelin-13 and apelin-17 are the preponderant circulating isoforms, while apelin-36 is also detected at a lower concentration close to 10% of apelin-13 level [10,11]. In rats, (Pyr^1)apelin-13 and apelin-17 are the most prevalent circulating isoforms [12]. While short apelin isoforms seem preponderant in the circulation, this is less clear in tissues. In rats, apelin-36 is preponderant in lung, testis and uterus but similar level of apelin-36 and apelin-13 are present in mammary gland [13]. (Pyr^1)apelin-13 is the predominant apelin isoform in human placental chorionic villi [14]. It remains to be determined how the proportion of apelin isoforms is fully regulated in circulation or tissues. The characterization and quantification of individual apelin isoform is only possible by mass spectrometry methods. However, most studies have assessed total apelin levels in tissues or circulation by enzyme- or radio-immunoassays (EIA or RIA) using antibodies targeted against the last 12 amino acids allowing detection of all apelin isoforms.

In adults, the apelin-APJ system plays a role in numerous physiological processes. Apelin favors angiogenesis, vasodilatory and hypotensive effects, cardiac contractility, diuresis, stress response, neuroprotection, glucose uptake, digestive motility and absorption, and satiety. For more information on the role of apelin-APJ system in these processes, we refer the reader to recent reviews [5,15–17]. Consistent with its multiple endocrine functions, circulating apelin level vary in response to multiple stimuli. While plasma hyper-osmolality, dehydration and hypoxia [10,18,19] increases plasma apelin levels, acute fasting reduced its concentration [20–22]. Apelinemia also follows a circadian rhythm in mouse, with increased concentration during the dark period (i.e. feeding period) [23]. Interestingly, alterations in circulating apelin levels have been reported in multiple pathologies such as type 2 diabetes (T2D), obesity, cardiovascular diseases, abnormal fluid homeostasis, cancer and tumor angiogenesis [24]. It has been suggested that apelin may be a therapeutic target in many metabolic diseases whereas, in cancers, apelin appears deleterious as it promotes angiogenic tumor growth [24,25].

Elabela (also referred to as Toddler, or Apela) was first identified in zebrafish embryos as a new ligand of APJ receptor [26,27]. The *elabela* gene was initially thought to produce a non-coding RNA but Chng et al. [26] and Pauli et al. [27] elegantly showed that this gene encodes a 54 amino acid preprotein processed in several isoforms: elabela-32, elabela-22, elabela-21 and elabela-11. *Elabela* is expressed in embryos but only in few adult tissues such as the cardiac endothelium, blood vessels, kidney, prostate and placenta [26,28–30]. As apelin, elabela also circulates in blood in human and mouse [29–32]. However further work is necessary to identify preponderant elabela isoforms and the mechanism (s) regulating their production.

3. Roles of Apelin, Elabela and APJ in pregnancy

3.1. Role in embryonic development

3.1.1. Elabela-APJ axis is involved in heart development

The role of APJ in cardiovascular development was first highlighted in zebrafish as most embryos carrying the *Grinch* mutation, localized to the APJ zebrafish ortholog, AGTRL1b, display no heart [33]. Moreover, knock-down of AGTRL1b in 1-cell embryos lead to higher lethality due to no heart or heart abnormalities [33–35]. Later, it was shown that APJ knock-out mice display partial embryonic lethality with more than half of the embryos dying at 10.5 days of gestation because of cardiovascular defects including deformed vasculature of the yolk sac and the embryo as well as poorly looped hearts with aberrant ventricles [6,7]. Moreover, most surviving embryos show cardiovascular defects in adulthood such as impaired vascular maturation and/or an abnormally formed myocardial trabeculation and ventricular wall development [6,7].

The discovery of elabela, in 2003, has helped reconcile the puzzling observation that while APJ-deficient animals exhibit partial embryonic lethality and cardiovascular defects, apelin-deficient animals are viable and fertile with no major heart defects [6,8,34–36]. These studies suggested, early on, that APJ had apelin-independent functions for heart development. Recent studies have shown that phenotypes caused by loss of elabela function in zebrafish and mouse are very similar to phenotypes induced by APJ deletion, i.e. partial embryonic lethality and cardiovascular defects including underdeveloped yolk sac vasculature and impaired cardiac tube looping [26,27,29,37]. APJ and elabela have very similar spatiotemporal expression pattern in embryo during development. They are both detected early during gastrulation and throughout the subsequent developmental stages [26,38] whereas apelin expression initiates only at the end of gastrulation [34]. Overall, these results indicate that elabela, via binding to APJ, is crucial for the early heart development. It was first shown that altered cardiac development caused by APJ deficiency was due to failure of myocardial progenitors to migrate to the heart-forming region and to differentiate properly during gastrulation [33–35]. Coherent with this study, elabela deficiency impairs cell movement during gastrulation and meso-endoderm differentiation during embryogenesis [26,27,39,40]. Differentiation of endodermal precursors is crucial for guiding the overlying cardiac progenitors toward the heart-forming region [26]. Overall, these results indicate that elabela, acting through APJ, is involved in endoderm differentiation and subsequent heart morphogenesis (Fig. 1). Finally, a recent study has shown that elabela-APJ pathway may have impact on the skeletal development, bone formation and homeostasis by modulating proportion of pluripotency factors in ventrolateral endodermal cells [41].

3.1.2. Elabela underlies self-renewal in embryonic stem cells independently of APJ

Elabela was shown to underlie self-renewal of mouse and human embryonic stem cells (ESCs) (Fig. 1), notably by activating the PI3k/Akt/mTORC1 pathway which is involved in protein translation and cell-cycle progression [42]. Although these cells do not express APJ receptor, it was postulated that effects of elabela on ESCs may involve its activity as a regulatory RNA [42,43]. Alternatively, it could involve another unknown receptor. Recently, G protein-coupled receptor 25 (GPR25) was shown to be activated by both apelin and elabela in zebrafish and pigeons [44]. However and surprisingly, this study also demonstrated that these peptides could not activate the human GPR25 under the same conditions. Thus, the presence of an additional receptor for elabela in mammalian vertebrates remains to be determined and further studies are needed to understand the role of GPR25 in non-mammalian vertebrates.

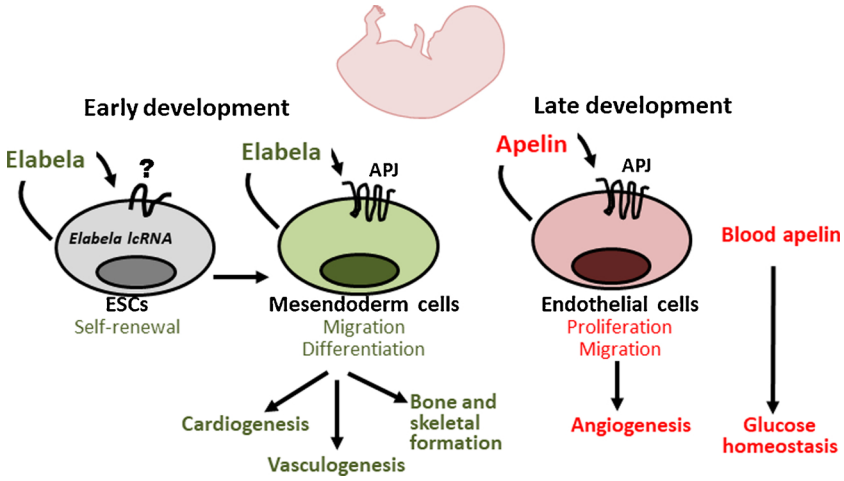

Fig. 1. Roles of elabela and apelin in embryonic development. Elabela actions (in green) are critical for early embryonic development. Elabela is highly expressed in embryonic stem cells and has been shown to underlie their self-renewal capacity. This effect is independent from APJ receptor and could involve a new unknown receptor or elabela acting as a regulatory RNA. Elabela-APJ axis has been shown to play a crucial role in cardiogenesis (early heart morphogenesis) and vasculogenesis (formation of primitive vascular networks) by facilitating the migration and differentiation of mesendodermic cells. Contrary to elabela, apelin appears to act later in embryonic development (in red). The APJ-apelin is mainly involved in angiogenesis (veins and arteries expansion) via endothelial cells proliferation and assembly. Furthermore, apelin-APJ system was proposed to control other fetal organ maturation and energy homeostasis.

3.1.3. Elabela/Apelin-APJ in embryonic vasculogenesis and angiogenesis

As highlighted before, animals deficient for APJ or elabela display vasculature defects [6,7,33–35]. Conversely, mice deficient for apelin are viable and fertile but they display a delay in retina and heart vascularization at birth [8,34]. Consistently, Cekmez et al. [45] have shown preterm newborns which display retinopathy have lower cord blood apelin levels than preterm newborns without retinopathy. APJ receptor is highly expressed in both endothelial precursor cells (angioblasts) and endothelial cells of the developing vasculature in mouse, frog and zebrafish embryos [38,46–48], suggesting a role of APJ in vasculogenesis and angiogenesis. While vasculogenesis is the process of formation of de novo primitive vascular networks directly from angioblasts, angiogenesis is a process of formation of new vascular segments by sprouting from the pre-existing vessels.

Recent studies have shown that elabela-APJ axis appears involved in vasculogenesis whereas apelin-APJ axis is mainly involved in embryonic angiogenesis (Fig. 1). Helker et al. [47] recently showed that angioblasts rely on their intrinsic expression of APJ for their migration in the midline in zebrafish. Further, they showed that angioblast migration is mainly triggered by elabela. Apelin is highly expressed in endothelial cells of embryonic vessels in frog embryos [49]. Cox et al. [49] have shown that apelin increased angiogenic branching in the frog embryo and the chicken chorioallantoic membrane system. Moreover, Kidoya et al. [48] have shown that the apelin-APJ system induces cell–cell assembly and the proliferation of vascular endothelial cells in mouse embryo. When the apelin gene was knocked out, the caliber of inter-somatic vessels in the embryo was narrower. These results indicated that the apelin-APJ system is involved in maturation of blood vessels by caliber size modification during angiogenesis [48].

3.1.4. Apelin-APJ in fetal maturation and energy homeostasis

Furthermore, apelin-APJ system was proposed to control other fetal organ maturation and energy homeostasis. Wang et al. [50] have shown higher apelin expression in fetal and postnatal rat stomachs than in adults. Apelin was shown to stimulate gastric cell proliferation in vitro [50]. Work from our group demonstrated that apelin administration at low doses to fetuses at embryonic day 21 (E21) obtained by caesarean section increases glucose uptake in fetal lung and muscles in an insulin-independent manner. Conversely, at higher concentrations, apelin administration reduces fetal insulinemia and increases fetal glycaemia [9] demonstrating an inhibitory role of high levels of circulating apelin on insulin release in the fetus. These data suggest that fetal circulating apelin may be implicated in energy metabolism and glucose homeostasis during fetal life. Interestingly, we have shown that a drastic maternal food-restriction during gestation reduces fetal apelinemia [9], suggesting that maternal nutritional state influence fetal apelinemia levels.

3.2. Role in placental formation and function

3.2.1. Elabela-APJ plays a role in early placental development

As previously mentioned, elabela is expressed in few adult tissues including the placenta. In mouse placenta, Ho et al. [29] demonstrated that elabela is first expressed in trophoblasts and is robustly up-regulated after allantoic fusion (an early phase of placental vascular development). By mid-gestation, elabela expression becomes restricted to another placental cell type, the syncytiotrophoblasts (STs). At this stage, elabela-positive STs are juxtaposed to APJ-expressing fetal endothelial cells, suggesting that elabela influences these cells in a paracrine manner. In human placenta, elabela is expressed in cytotrophoblasts (CTs) and STs all along the gestation [29], suggesting species differences in elabela function. Using human trophoblast-like choriocarcinoma cells, the authors have shown that exogenous elabela addition increased trophoblats invasiveness suggesting that elabela favors early placenta formation by favoring trophoblasts invasion into maternal uterine wall (Fig. 2) [29]. In accordance, elabela-deficient mice display smaller placentas than control mice, with thin placental labyrinths (area of feto-maternal exchange). Of note, placenta from elabela-deficient mice also displayed poor vascularization characterized by little angiogenic sprouting [29], suggesting that elabela favors placenta angiogenesis. This defect in placenta vascularization were shown to result from alterations in stalk versus tip cell populations [29], both important drivers of new blood vessel formation. While tip cells determine the direction of new angiogenic sprouts by extension of their filopodia, stalk cells proliferate to enable vascular sprout extension [51,52]. Because elabela-positive STs are juxtaposed to APJ-expressing fetal endothelial cells, it has been hypothesized that elabela has paracrine actions to favor angiogenic sprouting. Altogether, these data demonstrate that elabela is a key factor for early placental development and angiogenesis (Fig. 2).

In addition, Ho et al. [29] have shown that elabela is detected in maternal mouse serum during pregnancy, from embryonic day 7.5 (E7.5) to term with a peak at E12.5. Three recent studies in humans have reported that elabela is also present in serum of pregnant women [31,32,53]. Interestingly, chronic sub-cutaneous elabela treatment by osmotic pump in elabela-deficient pregnant mice normalizes their hypertension and proteinuria [29], suggesting that circulating elabela participate in maternal cardiovascular and renal adaptations to pregnancy. However, the release of elabela by the placenta or other maternal or fetal tissues into maternal blood remains to be fully demonstrated.

3.2.2. Apelin-APJ promotes placental vasodilatation and glucose transport

Contrary to elabela-deficient mice, apelin-deficient mice do not display any placental alterations in terms of vascular morphology [29],

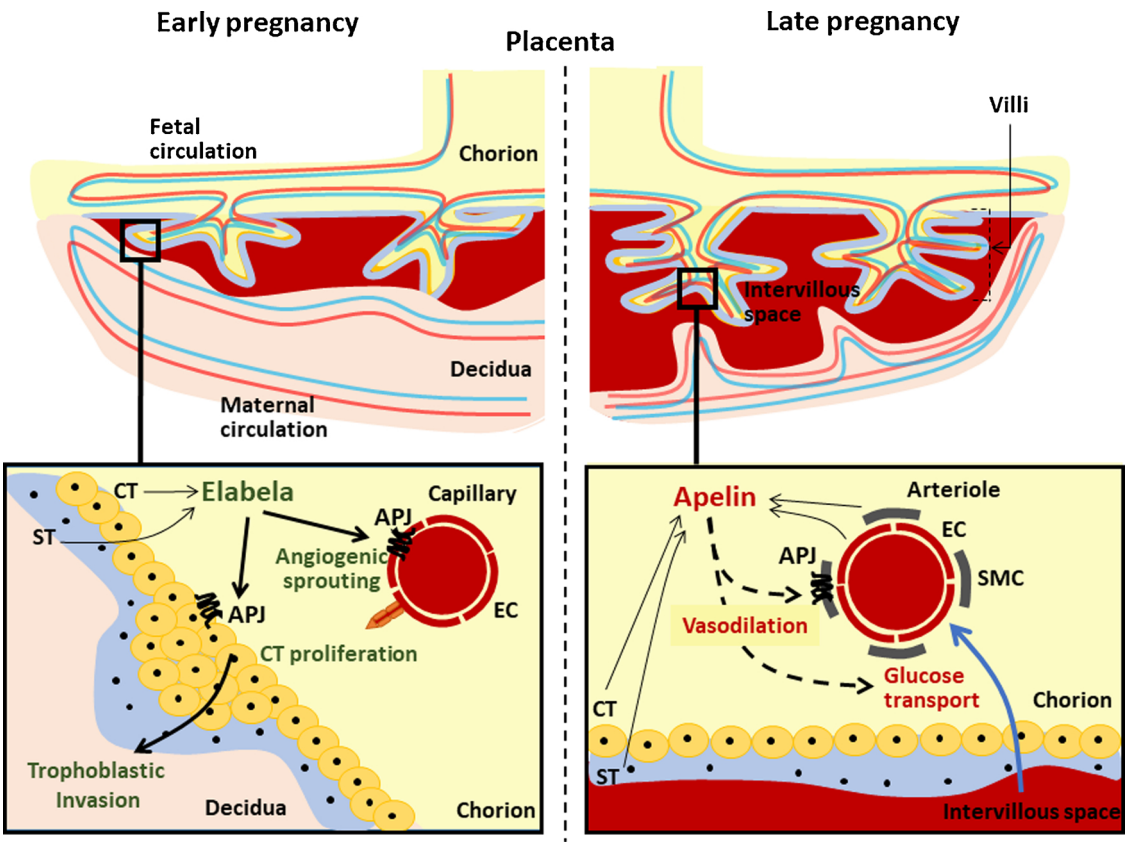

Fig. 2. Roles of elabela and apelin in placental development and function. The APJ receptor is expressed during all the gestation in numerous cells including cytotrophoblasts (CT), syncytiotrophoblasts (ST), endothelial cells (EC) and smooth muscle cells (SMC). **(A)** Elabela (in green) is critical for early placental development. In early gestation, elabela is produced by villous CT and ST. Elabela has been shown to promote: 1) CT proliferation and favor trophoblastic invasion within maternal decidua, and 2) fetal vessel angiogenesis. **(B)** In mid and late gestation, placental elabela expression is reduced while apelin expression is increased. Apelin (in red) is expressed in CT, ST, EC and SMC of fetal arterioles. At this stage, apelin-APJ signaling promotes vasodilation of fetal arterioles and glucose transport to the fetus.

indicating that apelin does not participate in placenta angiogenesis. Moreover, overexpression of apelin in elabela-deficient mice is not sufficient to rescue their placental defects, suggesting that these two APJ ligands elicit different signaling outcomes in the placenta [29]. Apelin (and APJ) is detected in numerous placental cells including CTs, STs, endothelial cells and smooth muscle cells of fetal arterioles [54,55]. Based on this broad distribution and its known vasodilatory function in the periphery [5], apelin may be implicated in placental vascular tone and influences subsequent maternal-fetal exchange of oxygen and nutrients (Fig. 2). We demonstrated that intravenous injection of apelin-13 to rat pregnant mothers increases the transplacental transport of glucose from mother to fetus, suggesting that placenta-derived apelin participates in fetal glycaemia (Fig. 2). Because this effect occurred without changes in the expression of main placental glucose transporters, GLUT1 and GLUT3 [9], we propose that apelin exerts its action through increased placental vasodilation. In agreement with this hypothesis, Dray et al. [56] demonstrated that apelin favors glucose uptake in muscle through NO-induced vasodilation. Moreover, Wang et al. [57] have shown that apelin treatment significantly ameliorated PE symptoms in rats by improving endothelial NO synthase/nitric oxide signaling. On the contrary, it was shown that the vasoconstrictor factor angiotensin II decreases apelin release from human placental villi explants, suggesting an opposite cross talk between the vasodilatory apelinergic system and the vasoconstrictor renin-angiotensin system (RAS) in the placenta [14].

3.2.3. The placenta as a regulator of fetal and maternal circulating apelin levels?

We and others have shown that explants from placenta release significant levels of apelin [9,14], and that circulating apelin levels is higher in fetus than in mothers in rodents and humans [9,58,59]. Interestingly, a drop in circulating apelin level is observed in newborns rapidly after birth [59], suggesting that the placenta could be a source of blood apelin for the fetus. The placenta could also participate to circulating apelin levels in the mothers although this is less clear. Maternal apelinemia tend to increase during gestation and drop significantly near term [9,58,60,61]. While apelin has been shown to be secreted by the placenta [9,14], other studies suggest that placenta could also participate in its clearance. Van Mieghem et al. [60] have shown that at term, the placenta display increased expression of angiotensin-converting enzyme-related carboxypeptidase-2 (ACE2) [60] that could contribute to the drop in maternal apelin levels at term. Indeed, this enzyme is able to catabolize and inactivate apelin peptides [62]. Finally, it remains to be investigated whether circulating apelin participates to maternal metabolic, cardiovascular and hydro-mineral adaptations to pregnancy.

4. Apelin and elabela in preeclamptic pregnancy

Preeclampsia (PE) is a serious disorder of pregnancy that is observed in 5–7% of all pregnancies worldwide [63]. PE accounts for almost 20% of pregnancy-related mortalities, and is associated with increased risk of premature birth and fetal growth restriction [63]. PE is characterized by hypertension beginning after 20 weeks of pregnancy, often

Table 1
Summary of studies examining the apelin-APJ system in pre-eclampsic (PE) and normal (N) patients. For each study, we indicated the cohort characteristics (sample size, age (in years), body mass index (BMI) and gestational age (in weeks) at time of measurement). All studies determined plasma apelin levels using the same Apelin-36 ELISA kit from Phoenix Pharmaceuticals which is said to cross-react with apelin-12, -13 and -36. Placental apelin and APJ level were determined by quantitative qPCR (mRNA) and/or immunohistochemistry (IHC), western blot (WB) or chromatography (HPLC). When known, IHC or WB antibodies' suppliers are indicated in parenthesis. Levels are either increased (↑), decreased (↓), unaffected (↔) or not available (na). Data are expressed as mean ± SD. Significant differences between N and PE patients are indicated as follow: $ $p < 0.05$ for maternal age, §$p < 0.05$, §§$p < 0.01$, §§§$p < 0.001$ for maternal BMI, #$p < 0.5$, ###$p < 0.001$ for gestational age, and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for maternal apelinemia.

Study	Sample size	Age (y)	BMI	Gestational age (w)	Maternal apelinemia (ng/ml)	Placenta	
						Apelin	APJ
Liao et al. 2007 [73] (China)	N (15) PE (36)	na	na	na	na	↓ mRNA ↓ protein (IHC)	na
Nishizawa et al. 2007 [74] (Japan)	N (24) PE (21)	29.2 ± 5.9 30.3 ± 3.7	22.9 ± 5.2 22.1 ± 3.1	32.9 ± 5.6 32.9 ± 4.0	na	↓ mRNA	na
Cobellis et al. 2007 [76] (Italy)	N (15) PE (15)	25 ± 0.6 30 ± 2.0	na	39 37	na	↑ protein (IHC, custom)	↑ protein (IHC, custom)
Furuya et al. 2012 [55] (Japan)	N (44)	na	na	23-40	na	↓ mRNA (early & late PE)	↓ mRNA & protein (early PE)
	severe PE (43)			24-41			↑/↔ mRNA & protein (late PE)
Bortoff et al. 2012 [70] (USA)	N (79) PE (76)	31.8 ± 5.1 29.5 ± 6.8 ($)	22.7 ± 3.8 27.2 ± 7.1 (§§§)	39.6 ± 1.3 35.6 ± 3.7 (###)	0.78 ± 0.31 0.66 ± 0.29 ↓(*)	na	na
Simsek et al. 2012 [78] (Turkey)	21 N (21) mild PE (31) severe PE (17)	32.0 ± 6.4 32.6 ± 3.3 28.5 ± 2.1	na	24-42	1.4 ± 0.7 2.0 ± 0.6 2.1 ± 0.6 ↑(**)	na	na
Inuzuka et al. 2013 [75] (Japan)	N (49)	31.6 ± 5.5	21.9 ± 4.1	35.4 ± 4.8	7.5	↓ mRNA	Trend to ↓ mRNA (p = 0.08)
	PE (47)	30.8 ± 4.5	22.3 ± 4.5	33.9 ± 3.1	9.0 ↑(***)	↓ protein (IHC, Abcam)	↓ protein (IHC, Assaybiotech)
Kucur et al. 2014 [77] (Turkey)	N (20) early PE (20)	29.3 ± 3.4 28.6 ± 6.1	29.1 ± 1.4 28.5 ± 2.3	29.3 ± 0.9 29.3 ± 2.1	5.7 ± 1.2 8.6 ± 3.6 ↑(**)	na	na
	N (20) late PE (20)	29.5 ± 2.3 29.2 ± 4.1	28.9 ± 0.6 29.1 ± 4.4	36.3 ± 2.1 36.0 ± 1.4	8.1 ± 1.8 9.6 ± 2.5 ↔	na	na
Yamaleyeva et al. 2015 [14] (USA)	N (22) PE (20)	23.6 ± 1.1 24.3 ± 1.3	31.1 ± 1.3 36.4 ± 2.1 (§)	38.2 ± 0.6 36.6 ± 0.6 (#)	na	↓ mRNA ↓ protein (HPLC)	↔ mRNA ↔ protein (IHC & WB, Merck)
Van Mieghem et al. 2016 [61] (USA)	N (8) PE (6)	36.9 ± 2.4 32.8 ± 3.9	25.8 ± 4.4 27.6 ± 9.2	36.6 ± 1.8 29.3 ± 3.7	1.7 ± 0.5 1.3 ± 0.3 ↔	↔ mRNA ↔ protein (IHC, Phoenix)	na
Colcimen et al. 2017 [71] (USA)	N (20) mild PE (20) severe PE (16)	25.2 ± 6.5 31.7 ± 6.9 28.7 ± 9.4 ($)	na	na	na	↑ protein (IHC, Abcam)	na
Pritchard et al. 2018 [31] (UK)	N (82) PE (82)	30.2 ± 1.2 29.6 ± 1.2	26.3 ± 1.0 26.7 ± 1.2 (§§)	40.4 ± 0.10 40.1 ± 0.35 (#)	na	↔ mRNA	na

accompanied by proteinuria and multi-organ dysfunctions. The clinical manifestations of PE are associated with general endothelium dysfunction such as vasoconstriction and organ ischemia [63–65]. Since the risk of maternal and fetal distress is particularly high in early onset PE, the fetus is often prematurely delivered between 32–34 weeks of gestation in this case [65,66]. The placenta of preeclamptic women is characterized by poor trophoblastic invasion and by endothelial vasospasm. These defects are thought to be the drivers of PE development [67]. Correct trophoblast invasion of the spiral arteries during implantation relies on a fine balance between placental angiogenic and anti-angiogenic factors. To date, PE development has been linked with the elevation of two placental anti-angiogenic factors, soluble Fms-like tyrosine kinase 1 (sFlt1 or sVEGFR-1) and endoglin. Indeed, administration of these factors to pregnant rats recapitulates PE-like symptoms [54,68,69].

4.1. Apelin-APJ is down-regulated in preeclamptic placentas

Apelin controls vessel tone in placenta. As such, several studies have investigated placental APJ and apelin expression in PE patients. Among all available studies (Table 1), some studies have used control and PE patient groups that were not matched for age, gestational age or body mass index (BMI) [14,31,70,71], or that included very small number of patients [61]. As apelin levels may vary with these factors [24,61,72], these limitations could introduce potential bias. Nevertheless, studies with adequate matching and group size have all reported a decrease in the placental mRNA expression of both apelin and APJ in PE patients [55,73–75] (Table 1). Three of these studies have confirmed a decrease in apelin and/or APJ at the protein level using immunohistochemistry (IHC) technics [55,73,75]. Only Cobellis et al. [76] have reported increased placental APJ by IHC. However these authors found nuclear and cytoplasmic localization of APJ which appears confusing given that APJ is a GPCR that should be localized primarily to the plasma membrane.

4.2. Maternal blood apelin is increased in preeclamptic pregnancies

A total of six studies have measured maternal plasma apelin levels in PE (Table 1) but two of them have either very low sample size [61] or differences between BMI and age between PE and control groups [70], introducing potential bias. Overall, maternal apelin levels are increased in PE versus control groups [75,77,78]. We propose that PE-associated maternal hypertension could induce apelin tissue and circulating levels to favor vasodilation in order to counteract maternal hypertension.

Table 2
Summary of studies examining the elabela-APJ system in pre-eclampsic (PE) and normal (N) patients. For each study, we indicated the cohort characteristics (sample size, age (in years), body mass index (BMI) and gestational age (in weeks) at time of measurement). Plasma elabela levels were determined in ng/ml using two distinct ELISA kits. Pritchard et al. [31] and Panaitescu et al. [32] used the Elabela human ELISA kit from Peninsula Laboratories which is said to react for elabela-32. Villie et al. [53] used the Human Elabela ELISA kit from Creative Diagnostics which cross-reacts with human elabela-21 and -32. Placental elabela and APJ mRNA levels were determined by quantitative PCR. For each studies, we indicated when levels are either increased (↑), unaffected (↔) or not available (na). Data are expressed as mean ± SD. Significant differences between N and PE patients are indicated as follow: $$ $p < 0.01$ for maternal age, § $p < 0.05$, §§ $p < 0.01$ for maternal BMI, #$p < 0.05$ for gestational age and ***$p < 0.001$ for elabela levels.

Study	Sample size	Age (y)	BMI	Gestational age (w)	Elabela	APJ
Pritchard et al. 2018 [31] (UK)	N (82)	30.2 ± 1.18	26.3 ± 1.05	40.4 ± 0.10	↔ mRNA	↔ mRNA
	PE (82)	29.6 ± 1.25	26.7 ± 1.25 (§§)	40.1 ± 0.35 (#)		
	N (32)	32.4 ± 1.52	25 ± 1.75	28.4 ± 0.92	20.5 ± 12.1	na
	PE (32)	32.7 ± 1.47	29 ± 2.25 (§§)	29.4 ± 0.87	28.5 ± 14.5 ↔	
Panaitescu et al. 2018 [32] (USA)	C (56)	24 ± 1.75	31.3 ± 2.52	30.3 ± 1.15	4.02 ± 1.06	na
	early PE (56)	24 ± 2.37	26.6 ± 2.32 (§)	30.4 ± 1.05	6.09 ± 1.96 ↔	
	C (60)	25 ± 1.75	28.5 ± 2.61	37.3 ± 0.59	4.17 ± 2.16	na
	late PE (57)	22 ± 1.87 ($$)	27.4 ± 2.74	37.3 ± 0.56	7.99 ± 2.05 ↑ (***)	
Villie et al. 2018 [53] (France)	N (14)	na	na	24.3 ± 1.28	8.71 ± 7.7	na
	PE (12)			24.8 ± 1.14	11.9 ± 10.8 ↔	

Table 3
Summary of studies examining the apelin-APJ system in gestational diabetes mellitus (GDM) and normal (N) patients. For each study, we indicated the cohort characteristics (sample size, age (in years), body mass index (BMI) and gestational age (in weeks) at time of measurement). When known, GDM-insulin dependant groups are indicated (GDM + I). Apelin levels were determined in maternal plasma and cord blood using radioimmunoassay (RIA) or enzyme-immunoassay (EIA) kits as indicated in the table, or by quantitative qPCR (mRNA) in placenta. Levels were either increased (↑), decreased (↓), unaffected (↔) or marked not available (na). Data are expressed as mean ± SD. Significant differences between N and GDM patients are indicated as follow: $$ $p < 0.01$ for maternal age and **$p < 0.01$, ***$p < 0.001$ for apelin level.

Study	Sample size	Age (y)	BMI	Gestational age (w)	Maternal apelin (ng/ml)	Cord blood apelin (ng/ml)	Placenta	
							Apelin	APJ
Telejko et al. 2010 [88] (Poland)	N (101)	30 ± 1.0	26.9 ± 1.0	28 ± 0.5	1.66 ± 0.07	na	na	na
	GDM (101)	31 ± 1.3	28.0 ± 1.1	28 ± 0.8	1.56 ± 0.09 ↔			
	N (16)	29.7 ± 1.7	31.8 ± 1.7	38.5 ± 0.75	1.53 ± 0.12	na	↔ mRNA	↔ mRNA
	GDM (20)	31.7 ± 1.2	32.7 ± 1.6	37.7 ± 0.75	1.61 ± 0.08 ↔ (RIA, Phoenix)			
Aslan et al. 2012 [92] (Turkey)	N (30)	31 ± 3.2	25.7 ± 2.8	36.7 ± 2.6	9.6 ± 5.9	8.2 ± 1.9	na	na
	GDM (30)	30.9 ± 4.2	25.9 ± 3.3	37.2 ± 2.4	13.5 ± 8.3 ↑ (***) (EIA, Phoenix)	8.8 ± 4.3 ↔		
Oncul et al. 2013 [89] (Turkey)	N (21)	na	na	na	0.16 ± 0.09	0.26 ± 0.13	na	na
	GDM (24)				0.14 ± 0.05 ↔	0.11 ± 0.03 ↓(***)		
Boyadzhieva et al. 2013 [93] (Bulgaria)	N (109)	30.6 ± 4.4	28.0 ± 5.4	24-28	7.97 ± 2.42	na	na	na
	GDM (127)	32.2 ± 5.2 ($$)	28.5 ± 6.6		6.89 ± 2.26 ↓ (**) (EIA, Phoenix)			
Cündübey et al. 2017 [90] (Turkey)	N (30)	na	na	38.5 ± 0.5	0.40 ± 0.07	0.46 ± 0.09	na	na
	GDM (25)			38.5 ± 0.3	0.40 ± 0.06	0.67 ± 0.19		
	GDM + I (27)			38.0 ± 0.3	0.51 ± 0.12 ↔ (EIA, Eastbiopharm)	0.47 ± 0.07 ↔		
Mouzaki et al. 2017 [91] (Greece)	N (44)	30.5 ± 4.5	27.9 ± 4.3	2nd trimester	0.93 ± 0.5	na	na	na
					1.2 ± 0.82 ↔			
	GDM (44)	31.0 ± 5.7	28.7 ± 6.6	3rd trimester	1.6 ± 2.5	na		
					1.4 ± 1.0 ↔ (EIA, Phoenix)			

However the origin of blood apelin in PE remains to be determined. Interestingly, Wang et al. [57] have recently shown that chronic apelin sub-cutaneous administration in a rat model of PE (reduced uterine perfusion) reduces maternal hypertension and proteinuria, and ameliorates fetal growth. This suggests that apelin could be a potential drug target for PE treatment.

4.3. Circulating elabela levels in preeclampsia

As previously mentioned, pregnant mice carrying elabela-deficient embryos display placental insufficiency of vascular origin and hallmarks of PE (hypertension, proteinuria, and glomerular endotheliosis) [29]. Moreover, subcutaneous injection of elabela between E11 to E19 to elabela-null mice prevented the development of maternal hypertension and proteinuria [29], suggesting a primary role of elabela in PE. To assess whether elabela may be involved in the etiology of PE in humans, three studies have recently measured circulating elabela levels in PE (Table 2). These studies revealed no difference in circulating elabela levels between PE and control patients [31,32,53] except in a group of women with late-onset PE [32]. To date, these studies do not support the hypothesis that human PE is characterized by an early deficiency in circulating elabela levels. Of note, using the same ELISA kit, two studies [31,32] found very different elabela concentrations in samples collected at similar time point, at term. Further studies are needed to

establish guidelines for adequate measurement of elabela as well as determine the relative variation of specific elabela isoforms.

5. Apelin in pregnancies with gestational diabetic mellitus and/or obesity

The apelin/APJ axis plays a role in the regulation of glucose homeostasis in adulthood by increasing glucose uptake and insulin sensitivity [15,56,79,80]. Studies have shown that individuals with type 2 diabetes (T2D) and obesity display increased circulating apelin levels [24,81,82], suggesting that apelinemia may be altered by defects in glucose and energy homeostasis. Gestational diabetes mellitus (GDM) is a serious pregnancy disorder, characterized by glucose intolerance and hyperglycemia that starts during pregnancy. It affects approximately 2–14% of all pregnancies worldwide depending on racial/ethnic group and the diagnostic test employed [83]. GDM increases the risks of numerous short- and long-term maternal and fetal complications such as PE, fetal macrosomia [84,85] or future T2D and cardiovascular diseases development [86]. Maternal obesity during pregnancy also increases risk of multiple maternal and fetal complications such as GDM, PE and preterm delivery [87]. We have shown that obese and insulin-resistant pregnant rat mothers display increased circulating and placental apelin levels at term [58], however no data are available in humans.

Six clinical studies have investigated the relationship between GDM and the apelin/APJ system (Table 3) and their findings suggest no association between apelin and GDM. Indeed, among available studies, four studies have reported no change in maternal plasma apelin between GDM and control patients [88–91] while one study found a significant increase [92] (Table 3). Another study found a small significant reduction in blood apelin levels in women with GDM [93], however controls were significantly different from GDM patients for age (Table 3). Some of these studies measured apelin levels in cord blood [89,90,92] but they also disagree. While circulating apelin levels are positively associated with T2D in adults, it seems not to be the case in pregnancies complicated with GDM. Our conclusions agree with a recent review assessing evidence for association between several circulating factors with GDM [94]. The presence of contradictory results for GDM may also underline the presence of confounding factors masking a clear result.

6. Conclusion and future directions

Current literature highlights the crucial role of the elabela/apelin-APJ systems in fetal and placental development. Elabela appears to have specific roles in early fetal development, particularly for cardiovascular system formation, while apelin function emerge later to control fetal angiogenesis and energy homeostasis. Similarly, elabela is essential for early placental development by favoring trophoblast invasion and angiogenic sprouting while apelin regulates constitutive functions such as placental vessel tone and nutrient exchange. Both hormones acts through a common receptor that is expressed in multiple cell types in fetus and placenta through the whole pregnancy. In the future, it will be important to identify what confers the specificity of action of each hormones such as actions independent of APJ receptor (as it has been recently identified for elabela), actions via new receptors or regulating ligands, or actions of each specific elabela/apelin isoforms. In addition, it may be crucial to define if elabela/apelin actions discovered in mouse models apply to human pregnancy. This should also help to conclude on the potential role of elabela/apelin in pathological pregnancies such as PE. Indeed, if elabela-deficient mice display all the features of PE, current clinical studies show no difference in elabela circulating levels between PE and controls patients which does not support a role in PE development or the use of elabela as early PE marker. Contrary to elabela, apelin circulating levels are increased in PE. Further prospective studies are needed to test apelinemia as an early PE marker. In that context, development of new tools and recommendation would be useful. For example, small peptide hormones such as elabela and apelin are known to be quickly degraded by protease cleavage, in particular in plasma [95]. Adequate sample collection and preservation are thus essential.

Conflict of interests

The authors declare that they have no competing interests. CK is co-founder of Enterosys S.A. (Labège, France).

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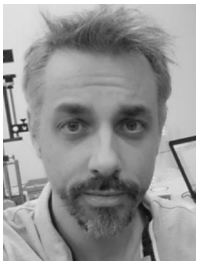

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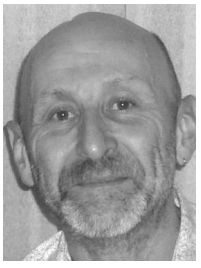

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