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STOX1: A new player in preeclampsia?

Eric M George[1] and **Gene L Bidwell**[2]

[1]Department of Physiology and Biophysics, The University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216

[2]Department of Neurology, The University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216

One of the most vexing and long-lasting conundrums in obstetrics is the underlying cause of preeclampsia. Preeclampsia is a hypertensive disorder of pregnancy which typically exhibits after the twentieth week of gestation, and is characterized by proteinuria, edema, an increased maternal inflammatory response, and widespread maternal vascular dysfunction. Unfortunately, despite intensive research into the pathophysiology of preeclampsia, effective therapeutic interventions for the disorder remain elusive. Currently, the only definitive treatment is parturition and removal of the placenta. It is this last fact which gives a clue to the pathological origins of the disease.

Extensive work over the last twenty years on both human subjects and experimental animal models has suggested that a central causative factor in the manifestation of the disorder is placental ischemia, brought about by inadequate remodeling of the maternal spiral arteries which supply the placenta. Under normal circumstances, invasive fetal cytotrophoblasts migrate into the spiral arteries, displacing the endogenous endothelial lining. In the process, they convert the arteries from small high-resistance vessels into distended high-capacity vessels. This process allows for adequate delivery of blood to the developing fetoplacental unit during gestation development. Failure of this process to proceed normally results in chronic hypoxia and ischemia(1). In response, the placental tissue begins producing factors which enter the maternal circulation and are responsible for the symptomatic phase of the disorder, in particular the anti-VEGF protein sFlt-1 and the soluble TGF-β receptor soluble endoglin (sEng)(2). What remains unclear, however, are the underlying reasons why cytotrophoblast invasion is commonly deficient in preeclamptic placentas. Several mechanisms have been proposed, ranging from maternal immune intolerance to genetic defects in trophoblast signaling. Several recent works have utilized placental microarray profiling to identify dysregulated genes and genetic screens, including genome-wide scans and linkage analyses, to identify mutated genes in the tissues of preeclampsia patients in the hopes of identifying these factors. One which has been a source of controversy in the literature is the transcription factor STOX1.

STOX1 is a winged helix transcription factor that was identified as a susceptibility gene for preeclampsia in a Dutch study of families in which two or more sisters were affected with

Address correspondence to: Eric M George, Ph.D. Instructor, Department of Physiology and Biophysics, University of Mississippi Medical Center, 2500 N. State St. Jackson, MS 39216, Phone: (601) 984-6689, Fax: (601) 984-1833, egeorge@umc.edu.

preeclampsia (3). van Dijk et al. found that the STOX1 gene was commonly mutated in preeclamptic patients, and affected sisters always harbored the same mutation. One mutation in STOX1, a missense mutation (Y153H), was predominant (3). Several subsequent *in vitro* and *ex vivo* studies added support for the role of STOX1 in preeclampsia. First, overexpression of STOX1 in choriocarcinoma cells resulted in a transcription profile that strongly correlated with transcriptomic alterations in placentas of preeclamptic patients (4). Also, α-T-catenin, a protein involved in cell-cell adhesion and known to be upregulated in preeclampsia, is highly induced by the Y153H mutant form of STOX1 (5). Overexpression of mutant STOX1 inhibits trophoblast invasion *in vitro* and STOX1 knockdown promotes outgrowth of extravillous trophoblasts in human first trimester placental explants. These effects of STOX1 are thought to be mediated by regulation of α-T-catenin (5).

Although the Dutch study found the STOX1 mutation to be highly associated with familial preeclampsia in its patient population, the mutation was subject to incomplete penetrance (3). Subsequently, other studies of more heterogeneous preeclamptic patient populations have failed to confirm the role of STOX1 in disease promotion. In a study of early onset, nonfamilial preeclampsia, Iglesias-Platas et al. found nearly all the STOX1 mutations identified in the van Dijk study as susceptibility mutations in both their control and affected populations, and they failed to observe a difference in STOX1 mutation frequency between control and preeclampsia patients (6). Similar results were found by Berends et al. in a study of 157 preeclamptic women and 157 controls in the Netherlands (7). In a large study of Finnish patients, no correlation was found between STOX1 single nucleotide polymorphisms (including the one causing the Y153H mutation) and preeclampsia, and no difference in STOX1 expression was found in the placentas of control and preeclamptic women (8). Curiously, the Finnish study also examined a subgroup of their patient population in which multiple women in the same family were affected, and again they were unable to identify STOX1 as a susceptibility marker (8). van Dijk et al. argue that in order to detect a susceptibility effect for the STOX1 Y153H mutation, only preeclamptic patients born to mothers who were also preeclamptic must be studied in comparison to non-preeclamptic and non-inherited preeclamptic controls (9).

In this issue, Doridot et al present data supporting a physiological role for STOX1 in the development of a preeclampsia-like state in mice. In this study, the investigators created transgenic mouse lines which significantly overexpress STOX1, particularly in the placenta. When wild type females were crossed with STOX1 homozygous knock-ins, they exhibited several symptoms which mimic symptoms of human preeclampsia. Perhaps most dramatically, systolic arterial pressure measured by tail cuff plethysmyography indicated a rapid increase in blood pressure from earliest gestation and reaching a plateau of ∼80mmHg increase by mid-gestation. Significantly, immediately after parturition, the systolic pressure immediately began a decrease to pre-gestational levels. Some pause should be given to these results, however, as confirmation of the pressure response by direct arterial measurements gave a somewhat more attenuated response. Additionally, the immediate increase in pressure exhibited in the STOX1 cross is in contrast to the human patient, which typically does not present with hypertension until mid-gestation or later. Similarly to preeclampsia however, crosses with STOX1 overexpressing males caused a significant elevation in proteinuria,

possibly due to observed alterations in renal structure, specifically capillary swelling and fibrin deposition, which could have an effect on renal hemodynamics and indicate renal injury. Perhaps most significantly, levels of sFlt-1 and sEng in the maternal circulation were also increased, suggesting a possible mechanism by which blood pressure could be increased, as both factors have been found to be important for the development of preeclampsia in other animal models. However, the levels of both factors demonstrated in these animals is significantly lower than that seen in the human population and other experimental models, suggesting that it is unlikely to be the major source of hypertension. One open question is what effect the STOX1 cross has on circulating VEGF and PlGF. This is important because the sFlt-1:VEGF/PlGF ratio, rather than total sFlt-1, is believed to be pathogenic as evidenced by the fact that the symptoms of sFlt-1 overproduction can be attenuated by exogenous VEGF or PlGF (10-12). Given the importance of the placenta in the development of preeclampsia, it is interesting that relatively minor alterations in placental morphology were observed. However, as the authors point out, the early increase in blood pressure, occurring prior to embryonic implantation, suggests that placentation leading to placental ischemia is unlikely to be a factor and it is likely a direct effect of the STOX1 expression on placental tissue which causes production of pathological agents. It also strengthens the case that the blood pressure effect of STOX1 overexpression is a result of direct action of STOX1-regulated genes acting in synergy with pregnancy-specific factors, such as maternal hormones.

Perhaps most interestingly, the authors found that administration of low-dose aspirin to the STOX1-crossed mothers resulted in a significant attenuation of both the maternal hypertension and renal fibrin deposition. It also had an intriguing effect on both fetal survival and weight. STOX1 crossed pregnancies exhibited a slight but significant decrease in litter size, agreeable with other reported models of preeclampsia, which was normalized by aspirin administration. The use of aspirin for the treatment and/or prevention of preeclampsia has been debated for some time in the literature, with support both for and against aspirin treatment extant (13-14). The results of this study suggest that aspirin administration could be an effective therapeutic for some of the pathogenic pathways in preeclampsia which could be mediated by STOX1.

One problem inherent in preeclampsia research is a relative dearth of adequate animal models. There are no reports in the literature of spontaneous preeclampsia in any organism outside of humans and limited genetic mimetics, such as the well-characterized BPH/5 mouse, which shares several of the symptoms of preeclampsia (15). Infusion or induction of specific proteins, like sFlt-1, have proved useful, but only partially recapitulate the total phenotype (16-17). The most successful models of preeclampsia symptoms have come from studies which utilize mechanical constriction of the arteries supplying the uteroplacental unit, causing chronic ischemia and closely mimicking the phenotype of severe preeclampsia, including hypertension, angiogenic imbalance, oxidative stress, and autoimmunity(18-20). While the results of this study suggest several aspects of preeclampsia symptoms, it will be illuminating to see whether these other pathways are activated. Should it prove to be so, STOX1 overexpression could prove to be a useful surrogate model for preeclampsia research. While the effect of STOX1 overexpression on placental perfusion remains unclear, the relatively minor effect of STOX1 on placental morphology suggests it is more likely that

STOX1 is having a direct transcriptional effect on pathogenic genes. As there is currently very limited information as to what genes are regulated by STOX1, further mechanistic studies looking at the direct role of STOX1 on genes known to be pathogenic in preeclampsia, should prove enlightening.

While the linkage between STOX1 and preeclampsia in human populations remains controversial, the data presented in this study suggests an intriguing link between STOX1 and gestational hypertension. It also suggests the exciting possibility that STOX1 overexpression could be a novel experimental preeclampsia model. Future work examining the role of STOX1 overexpression on other pathogenic pathways and its possible dysregulation in other experimental forms of gestational hypertension will tell whether STOX1 overexpression will prove a useful new model of human preeclampsia.

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