44 FETAL ORIGIN OF ADULT DISEASES: GENETIC IMPRINTING VERSUS DEVELOPMENTAL PROGRAMMING

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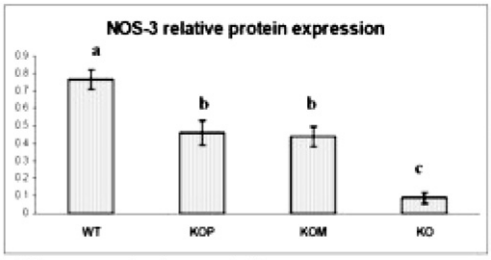
OBJECTIVE: We have previously shown that heterozygous mice offspring born to transgenic mothers lacking endothelial nitric oxide synthase (NOS3) have altered vascular function in later life compared to offspring born to wild type mothers. Our objective was to differentiate between a genomic versus uterine environmental etiology by characterizing the expression of NOS-3 protein in the vasculature of the offspring.

STUDY DESIGN: Homozygous NOS3 knockout (KO) and wild type mice (WT) were cross-bred producing maternally-derived (KOM; n=13) and paternally-derived (KOP; n=11 heterozygous litters. KO (n=4) and WT (n=10) litters were also obtained for control. Animals were genotyped by PCR using DNA isolated from their tail. Offspring were sacrificed at 14 weeks of age. Aortas were isolated from all groups, homogenized and protein extracted for Western blot analysis. NOS-3 was detected using a monoclonal antibody and protein expression was normalized to beta-actin. Data are presented as mean±SEM. Student t test and ANOVA were used for statistical analysis as appropriate (significance $P<0.05$).

RESULTS: Expression of NOS-3 was confirmed by the presence of a single band at 140 KD. NOS-3 protein expression was higher in WT compared to KOP ($p=0.003$), KOM ($p=0.0004$), and KO ($p<0.0001$). There was no difference between KOP and KOM ($p=0.83$). Data in figure presented as mean±±SE.



* different superscripts denotes p≤0.001

CONCLUSION: The difference in vascular phenotype previously reported in heterozygous NOS-3 offspring born to KO versus WT mothers is not mediated by different expression of the NOS-3 gene. This favors the role of the adverse intrauterine environment induced by NOS3 deficiency in the mother, rather than genetic imprinting. Along with prior findings, our results confirm the role of the uterine environment in the developmental origin of adult diseases and underscore the potential to significantly impact long term health by improving the intrauterine environment.

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45 URINARY ISOPROSTANE LEVELS SHOW A BIPHASIC PATTERN IN PATIENTS WHO DEVELOP PREECLAMPSIA

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OBJECTIVE: Early implantation events are modulated by reactive oxygen species and these are thought to play an important role in preeclampsia. Measurement of urinary isoprostanes is a reliable indicator of free radical activity in vivo and may offer insight into the etiopathogenesis of preeclampsia. Literature to date report conflicting results regarding the relationship of isoprostane levels to preeclampsia. Our hypothesis is that increased activity of reactive oxygen species occurs early in those pregnancies that later develop preeclampsia

STUDY DESIGN: 524 women were recruited as part of the Behavior in Pregnancy Study (BIPS). This cohort was followed until delivery and 24 hour urine sample collections performed at three time intervals (T1: 18-20 weeks, T2: 28 – 30 weeks and T3: 35 – 36 weeks). 20 patients were studied who were initially normotensive at T1 but subsequently developed preeclampsia.In a retrospective fashion, the urine samples from these were assayed by ELISA for 15-F2t-IsoP using a commercially available kit (Oxford Biomedical Research, Michigan) and compared with samples from matched controls

RESULTS: Table:

CONCLUSION: Elevated urinary isoprostanes at 18-20 weeks of pregnancy are associated with the subsequent development of preeclampsia. Beyond 28 weeks of gestation, urinary 15 F2t-Isoprostane levels were significantly lower in subjects who developed preeclampsia than controls who showed a continued modest increase in levels. This biphasic pattern may explain the apparent conflicts in reported literature. The late decline may be a response to higher than normal oxygen tensions at the maternal–fetal interphase in preeclamptic pregnancies which suppresses maternal isoprostane production as an adaptive response

Urinary 15 F-2-t isoprostane levels (pg/ml) by gestation

	Subjects (n=20)	Controls(n=22)	p-value
T1 (18-20weeks)	3018	1869	<0.001
T2 (28-30weeks)	1436	2203	<0.001
T3 (35-36 weeks)	1824	2470	<0.001

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46 PRESENCE OF MISFOLDED SOLUBLE OLIGOMERS IN URINE OF WOMEN WITH SEVERE PREECLAMPSIA CLASSIFIES PREECLAMPSIA AS A PROTEIN CONFORMATIONAL DISORDER

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OBJECTIVE: Protein conformational disorders such as Alzheimer's, light chain amyloidosis and prion diseases are propagated by amyloid fibril formation and aggregation due to defective folding of cellular proteins into aberrant 3D structures. A novel observation is that soluble pre-amyloid oligomers (intermediates in fibril assembly) have proteotoxic effects leading to endothelial damage and oxidative stress. As these processes play pathogenic roles in severe preeclampsia (sPE), our aim was to identify and characterize the nature of urinary soluble pre-amyloid oligomers in this pregnancy-specific condition.

STUDY DESIGN: We analyzed 111 urine samples from women enrolled in 3 groups: sPE (n=49, GA: 28±1 wks), chronic hypertension (cHTN n=12, GA: 29±1 wks) and normotensive controls (CRL n=50, GA: 28±1 wks). Equal amounts of urine protein was subjected to dot blot using 3 conformation-specific antibodies recognizing prefibrillar soluble oligomers (***A11***), ring-shaped protofibrils (***Officer***) or fibrils (***OC***). Specificity was confirmed by omitting the primary antibodies. Identity of aggregated component proteins was sought by mass spectrometry and validated by Western blot with sequence-specific antibodies.

RESULTS: 1) Women with sPE had increased ***A11*** and ***Officer*** (sPE: 42±8 vs. cHTN: 9±6 vs. CRL: 3±1 U/μg, $P<0.001$) but not ***OC*** urine immunoreactivity, independent of GA; **2**) Urine ***A11*** and ***Officer*** dot blot staining intensity correlated with severity of hypertension ($P=0.007$) and proteinuria ($P=0.005$); **3**) Soluble oligomers in sPE urine were identified as light and heavy immunoglobulin chains, ceruloplasmin and the newly described 6-16 protein (G1P3), which is known to interact with Alzheimer's presenilin-2 protein to regulate apoptosis.

CONCLUSION: This is the first observation that PE is a conformational disorder characterized by amyloid-like assembly of proteins. Concurrent ***A11*** and ***Officer*** staining suggests that the misfolded intermediates have a propensity to assemble into pore-like structures (amyloid channels) that may play a role in clinical disease manifestations.

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47 TRANSFORMING GROWTH FACTOR-3 POLYMORPHISMS ARE ASSOCIATED WITH PREECLAMPSIA (PE)

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OBJECTIVE: Several lines of evidence indicate that O2–mediated downregulation of TGF-β3 is defective in women with PE, inhibiting early trophoblast differentiation. Polymorphisms of the TGF-β3 gene have not been studied in PE.

STUDY DESIGN: Cases of PE and controls were recruited retrospectively from delivery logs or during postpartum hospital stays from 1999-2008. Controls were matched to cases on maternal age and gestational age at delivery. DNA was collected from mothers and infants and a risk factor questionnaire was completed. Four SNPs were selected and genotyped using TaqMan assays. Statistical analysis was conducted using Stata 9.2. For all SNPs, homozygotes for the rare allele were collapsed with heterozygotes to increase statistical power. Logistic regression was used to model the association between genotypes and PE risk, adjusting for maternal age and gestational age at delivery.

RESULTS: Genotype frequencies among controls did not depart from Hardy-Weinberg equilibrium and the rate of genotyping failure was below 2% in all polymorphisms. In general, offspring carrying one or two copies of the rare allele were at increased risk of developing PE. Specifically, babies who carried the A allele (rs3917200), the C allele (rs2268624), or the T allele (rs2205181, rs11466414) had between a 50% and more than 300% increase in risk of being born to a preeclamptic mother than those not carrying that allele (OR=3.2, 1.8-5.6; OR=2.5, 1.5-4.1; OR=1.5, 0.9-2.8; OR=1.7, 1.0-2.9, respectively). All offspring associations were statistically significant except for rs2205181, which exhibited the same trend toward increased risk but was not statistically significant. None of the maternal genotypes were associated with PE risk, but the A allele (rs3917200) showed some suggestion of increased risk for PE (OR=1.6, 0.9-3.8).

CONCLUSION: These results are consistent with our understanding of placentation in that genetic variations that may result in placental dysfunction and subsequent preeclampsia would likely be of fetal origin.

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