MODIFIER GENES IN THE PATHOGENESIS OF CONGENITAL HEART DISEASE.

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Introduction. Mutations of the transcription factor Nkx2-5 cause serious congenital heart disease (CHD) in man, but only minor abnormalities have been reported in heterozygous knockout mice (Nkx2-5^{+/-}). The incidental discovery of a large VSD in one mouse suggested the hypothesis that Nkx2-5^{+/-} mice do develop significant CHD, the pathogenesis of which is determined in part by modifier genes.

Methods. Nkx2-5^{+/-} mice in the C57Bl/6 background were crossed to the FVB/n or A/J strain to test for effects of mixed genetic backgrounds. The F1 progeny were intercrossed or crossed back to C57Bl/6 to determine patterns of inheritance of modifier genes in the F2 progeny. Neonatal hearts were serially sectioned to identify congenital heart defects.

Results. Nkx2-5^{+/-} and wild-type mice in the C57Bl/6 background deviated from the expected Mendelian distribution (n = 654; p = 0.003); 15–20% of the heterozygotes were missing at weaning. Of 23 Nkx2-5^{+/-} C57Bl/6 near-term or newborn hearts two had moderate to large VSDs, and one had aortic coarctation, which would cause perinatal lethality. Five had ASDs. In contrast, the exact Mendelian ratio was obtained from a C57Bl/6 x FVB/n cross (N=545), and Nkx2-5^{+/-} F1 hearts were all structurally normal (N=29). In the C57Bl/6 x A/J F1 progeny, 2/41 Nkx2-5^{+/-} hearts had ASDs. In the C57Bl/6 x FVB/n F2 back- and intercrosses, 8 VSDs but no ASDs were found among ~150 Nkx2-5^{+/-} hearts. In the C57Bl/6 x A/J F2 back- and intercrosses, 2 VSDs but no ASDs were found among 28 hearts. Wild-type hearts in all strain backgrounds were normal (N=50).

Conclusion. Nkx2-5 mutation can cause significant CHD in humans and mice. Inbred strain crosses indicate that modifier genes affect the pathogenesis. The results suggest that homozygosity of a C57Bl/6 allele, which is polymorphic in FVB/n, contributes to the development of VSDs, whereas multiple modifier genes are involved in ASDs. The same or an entirely different VSD locus may also be polymorphic between C57Bl/6 and A/J. The ASD modifiers are polymorphic between C57Bl/6 and FVB/n and identical or similar between C57Bl/6 and A/J. The relative number of genes involved can explain why VSDs are more common than ASDs or other types of heart defects in man.

IMPACT OF HYPOXIA ON EARLY CHICK EMBRYO GROWTH AND CARDIOVASCULAR FUNCTION.

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Oxygen tension is a critical factor for appropriate embryonic and fetal development. Chronic hypoxia exposure alters cardiovascular (CV) function and structure in the late fetus and newborn, yet the immature myocardium is considered to be less sensitive to hypoxia than the mature heart. We tested the hypothesis that hypoxia during the period of primary CV morphogenesis impairs immature embryonic CV function and embryo growth. We incubated fertile white Leghorn chick embryos in 15% oxygen (hypoxia) or 21% oxygen (control) until Hamburger-Hamilton stage 21 (4 days). We assessed *in ovo* viability and dysmorphic features and then measured ventricular pressure and dimensions and dorsal aortic arterial impedance at stage 21. Chronic hypoxia decreased viability and embryonic wet weight. Chronic hypoxia did not alter heart rate or the ventricular diastolic indices of end-diastolic pressure, maximum ventricular -dP/dt, or tau. Chronic hypoxia decreased maximum ventricular +dP/dt and peak pressure, increased ventricular end-systolic volume, and decreased ventricular ejection fraction, consistent with depressed systolic function. Arterial afterload (peripheral resistance) increased and both dorsal aortic stroke volume and steady-state hydraulic power decreased in response to hypoxia. Thus, reduced oxygen tension during early cardiac development depresses ventricular function, increases ventricular impedance (afterload), delays growth, and decreases embryo survival, suggesting that a critical threshold of oxygen tension is required to support morphogenesis and cardiovascular function in the early embryo.

COMPARISON OF IMPEDANCE CARDIOGRAPHY TO DIRECT FICK AND ECHOCARDIOGRAPHIC MEASUREMENT OF CARDIAC INDEX IN CHILDREN.

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BACKGROUND: Impedance cardiography (ICG) provides a continuous, noninvasive measurement of cardiac index (CI) that has been useful in the intensive care setting in adults. However, there is limited data in its application to children. The purpose of this study was to validate a now commercially available ICG system in children.

METHODS: Seventeen patients (58% male; mean age 4.2 ± 5.2 years) with biventricular circulation without significant residual shunting were prospectively studied during cardiac catheterization or post-operative recovery in the intensive care unit. Oxygen consumption was measured by indirect calorimetry, while mixed venous and arterial saturations were drawn from existing catheters. Echo measurement of CI was determined by pulsed Doppler and 2-D diameter measurement of the left ventricular outflow tract. Serial ICG and echo CI measurements were performed simultaneously with Fick measurement while the patient was hemodynamically stable. **RESULTS:** The intraclass correlation coefficient, a measure of reproducibility, for ICG and echo was 0.97 and 0.88, respectively. CI correlation was as follows: Fick vs. ICG (r = 0.82), echo vs. ICG (r = 0.33), and Fick vs. echo (r = 0.34). The mean Fick CI measurement was 3.95 ± 1.54 L/min/m². ICG underestimated Fick and echo CI measurement on average by 1.02 ± 0.89 and 0.97 ± 1.65 L/min/m², respectively.

CONCLUSIONS: Serial ICG measurements of CI have better reproducibility and better correlation to the direct Fick method when compared to echo measurements in children. In conclusion, ICG is a reliable tool of predicting CI in children with the advantage of being technically easy, continuous, and noninvasive.

RETROSPECTIVE SCREENING FOR HYPERTENSION/PRE-HYPERTENSION IN A PEDIATRIC SUB-SPECIALTY CLINIC.

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The 2004 NHBPEP report provided updated normative data for systolic and diastolic blood pressure (SBP/DBP) in children. These data now define pre-hypertension (Pre-HTN) as well as HTN for gender, age, & height. Pediatric Endocrinologists see many diagnoses that may impact BP. **Purpose:** To retrospectively review and screen BP measurements, using 2004 NHBPEP data, for patients (pts) attending an out-pt, University-based, Pediatric Endocrinology Clinic.

Methods: Chart review of visits (7/1/04–12/31/04) by the MD or NP for pts between 1–20 years of age. BP typically was obtained from the right arm manually or using a Critikon Dinamap™ 8100T monitor. Recorded gender/age/height/weight values subsequently were entered onto a PDA with the Stat Growth-BP™ program where pts’ BPs then were noted as NL, Pre-HTN, or HTN. Pre-HTN = SBP +/or DBP between 90–95%; HTN = SBP +/or DBP ≥95%.

Results: (Table). Excluded pts: 24 exceeded age range; 75 visits were lacking height, weight, or BP.

	# of Visits	? / ?	# of Pts	? / ?	? / ? Age (yrs; X± SD)
Total Scheduled Visits	989	NA	NA	NA	NA
Total Actual Visits	881	NA	NA	NA	NA
Total @ Criteria	782	414 / 368	642	345 / 297	11.0 ±4.4 / 11.9 ±4.8
⊕ HTN	200	116 / 84	181*	106 / 75	11.6 ±4.7 / 11.7 ±4.9
⊕ Pre-HTN	83	45 / 38	80	43 / 37	12.2 ±3.6 / 11.3 ±4.9

*30 pts had NL BP on another visit; 17 pts were diagnosed previously with HTN; 5 pts were noted as agitated or upset during BP measurement.

Summary: Initial review of retrospectively collected data found 164 [(181–17) of 642; 25.5%] previously undiagnosed pts who screened positive for HTN on ≥ 1 occasion; 80 of 642 pts (12.5%) fulfilled new criteria for Pre-HTN.

Discussion: The origins of adult cardiovascular disease often lay in childhood; pts frequently seen in an Endocrinology clinic (e.g. for diabetes mellitus, obesity, dysmetabolic syndrome, sexual precocity, adrenal & thyroid disorders) may be especially at risk. *By definition*, HTN is confirmed with ≥ 3 elevated SBP +/or DBP readings.

Conclusions/Speculations: In a single University-based Pediatric Endocrine clinic, many patients with borderline or elevated BP are under-appreciated. A general pediatric clinic may be much busier. Clinicians must employ an efficient method to correctly identify HTN & Pre-HTN in children, relative to gender, age, and height.

SINGLE NUCLEOTIDE POLYMORPHISMS IN SMOOTH MUSCLE REGULATION GENES AS RISK FACTORS FOR SPONTANEOUS PRETERM DELIVERY.

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Preterm birth is a major cause of infant morbidity and mortality on the United States, occurring in 12% of all pregnancies. The aim of this study was to use single nucleotide polymorphisms (SNPs) in genes that regulate smooth muscle activity to determine if specific allelic variants in one or more genes may contribute to an increased risk of preterm delivery. DNA was extracted from cord blood of babies admitted to the NICU at the Children’s Hospital of Iowa, as well as their parents when available. A total of 14 SNPs from 8 different genes (PTGES, PTGES2, EDN1, NOS2A, PTGER1, PTGER2, PTGER4, and HPGD) were initially evaluated. Genotypes were determined using the PCR-based TaqMan assay platform, commercially available from Applied Biosystems, and analyzed on an ABI 7900 HT. A family-based association test was performed on genotyping data to evaluate for the presence of over-transmission of alleles of interest. A total of 392 infant samples were genotyped. 179 of these were from complete proband/parent triads while another 122 from were proband/parent diads. The remaining 91 were infants only. Gestational ages of the infants ranged from 22 weeks to 36 weeks with the mean gestational age being 31.2 ± 3.6 weeks. Significant results, defined as a p-value of less than 0.05 were found for markers in prostaglandin E synthase1 (PTGES; p=0.00198), endothelin 1 (EDN1; p=0.0114), and nitric oxide synthase 2A (NOS2A; p=0.033). Our data suggests that there are SNPs in genes that regulate smooth muscle contraction moderated through the fetus that may act as markers for increased risk of preterm delivery. Once validated these markers may be used to identify high-risk pregnancies, plan preventative strategies and look for new drug targets for improved tocolysis.