Program/Abstract # 311  
Mesenchymal β-catenin regulates Tbx1 expression and causes DiGeorge-like phenotypes  
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DiGeorge syndrome is one of the most common genetic diseases in humans affecting 1 in 4000 people. This disease is characterized by pharyngeal apparatus malformations causing outflow tract alignment and cardiac septation defects, thymus, parathyroid aplasia/hypoplasia, and craniofacial defects. Most cases of DiGeorge syndrome are caused by deletion of chromosome 22q11 and Tbx1 is the candidate gene for cause of the disease. Tbx1 heterozygous mice show minor cardiovascular defects. Whereas, Tbx1 null mice display the most severe features of the disease. Tbx1 over-expressing mice also exhibit DiGeorge-like phenotypes, indicating that the gene dosage of Tbx1 is critical. Here, we show that canonical Wnt/β-catenin signaling negatively regulates the Tbx1 gene and that mesenchymal deletion of β-catenin using Dermo1-cre causes a DiGeorge-like phenotype. Phenotypes include, abnormalities of the great vessels, including aberrant emergence of the right and left subclavian arteries, hypoplastic pulmonary arteries, aortic arch hypoplasia, major cardiac outflow tract abnormalities classified as double outlet right ventricle, overriding aorta, pulmonary truncus arteriosus, ventricle septation defect, atrial septation defects, micrognathia, thymus hypoplasia and detachment. Tbx1 expression is up-regulated in β-catenin conditional knockout embryos. Fgf8 expression and the FGF8/FGFR signaling downstream targets Erm and Pea3 are also increased. This finding indicates that Wnt/β-catenin signaling is important for modulating the level of Tbx1 expression and that disruption of this balance is a likely etiology of DiGeorge-like disease phenotypes.

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Program/Abstract # 312  
The effect of embryo biopsy on gene expression and development in the preimplantation mouse embryo  
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Preimplantation Genetic Diagnosis (PGD) is a form of prenatal screening done on embryos prior to the initiation of pregnancy so that only select embryos are transferred. PGD requires assisted reproduction, culture to the 8-cell stage, and an invasive embryo biopsy procedure which involves: 1) incubating embryos in divalent-cation-deficient medium to disrupt cell adhesion, 2) breaching the zona pellucida with Acidic Tyrode’s, laser drilling, or mechanical force and 3) aspirating one or two blastomeres. Although PGD has been used successfully in the clinic, the risks associated with PGD for the health and well-being of the offspring have not been examined in an animal model. In this study we developed a mouse model of PGD to determine the effect of various aspects of the biopsy procedure (incubation in Ca²⁺/Mg²⁺-free medium, Acidic Tyrode’s treatment, blastomere aspiration), performed individually or in combination, on preimplantation embryo development and global patterns of gene expression. There was no significant difference between the treatment groups in terms of preimplantation embryo development. However, a significant percentage of embryos that were subject to Acidic Tyrode’s treatment hatched prematurely. Microarray analysis demonstrated that the treatment groups were more similar than different in terms of global gene expression. Multiple Statistical Analysis of microarray analysis showed that no genes were different among the treatment groups. These results suggest that there is not a correlation between the embryo biopsy procedure and alterations in preimplantation development and global gene expression.

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Program/Abstract # 313  
Role of MESD in WNT signaling and lipoprotein metabolism  
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Loss of mesd (mesoderm development) blocks gastrulation and mesoderm differentiation in mice. Polarity defects likely result from improper localization of WNT co-receptors LRP5/6. LRP5/6 are members of the LRP (low-density lipoprotein related receptors) family. We hypothesize that MESD functions more broadly to fold LRPs. Consistent with this hypothesis, mesd mutants are smaller than wnt3a−/− or lr p5/6−/− mutants. Using a cell culture secretion assay, we show that MESD is required for trafficking LRPs containing a beta-propeller/EGF motif. In mesd mutants, the scavenger receptor LR2 (megalin) is diffusely localized in the VE of mesd mutants compared to apical localization in wt embryos. LR2 is important for nutrient uptake in the visceral endoderm (VE), as well as for protein clearance by the kidney proximal tubule. Electron micrographs show that the VE in mesd mutants has a reduced number of vesicles, as compared to wild-type littermates. Further, uptake of dl-HDL is impaired in mesd mutants. This suggests that the growth defects result from impaired nutrient uptake as a result of improperly localized LRPs. If MESD is a general LRP family chaperone, MESD may have an additional, and novel, role as a regulator of cardiovascular health. LR family members LR1, LDLR, and VLDLR have
previously been shown to be important in regulating lipoprotein metabolism. Using a floxed allele of mesd, we are examining the effects of tissue-specific loss of MESD on plasma lipoprotein levels and arterial plaque formation. This work was supported by GM5396407 to BCH.

Program/Abstract # 314
A zebrafish genetic model of spinal muscular atrophy and functional analysis of the Smn-binding protein, Gemini2
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Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by a loss of α-motoneurons in the spinal cord. SMA is caused by low levels of the ubiquitously expressed Survival Motor Neuron (Smn) protein. In previous studies, we have shown that morpholino (MO) knockdown of smn in zebrafish embryos results in motor axon-specific outgrowth defects. To obtain a genetic zebrafish model of this disease, we have identified 3 smn mutations by TILLING. smnc−/− zebrafish have high maternal Smn protein; at 11 dpf, however, protein levels are undetectable and the larvae die at 11–12 dpf. Synapse analysis reveals NMJ changes consistent with denervation in mutants (and morphants). To address the function of Smn, we knocked down Gemini2, a Smn-binding protein involved in snRNP assembly. gemin2 MO knockdown in the entire embryo showed overall abnormal development. However, when we knocked down Gemini2 specifically in motoneurons (either by iontophoresis or blastula transplantation), their axons developed normally. These data show that reduction of Gemini2, unlike Smn, does not directly cause motor axon outgrowth defects, which is consistent with an snRNP-independent role for Smn in motoneurons. Funding sources include: Families of SMA grants MCW2006 (MLM) and BOO2008 (KLB); NIH grants RO1NS50414 (CEB) and P30-NS045758.

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Program/Abstract # 316
Seeking the biochemical basis of type III 3-methylglutaconic aciduria through zebrafish models
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Type III 3-methylglutaconic aciduria (MGA-III) is a rare disorder with neuro-ophthalmic manifestations and increased urinary excretion of 3-methylglutaric acid (3MGA). Two familial mutations have been found in the OPA3 gene associated with MGA-III. We found that the zebrafish Opa3 orthologue is expressed ubiquitously during embryogenesis and is enriched in the brain from the pharyngula stage until at least 120 hpf. Antisense-based depletion of zebrafish Opa3 causes the signature increase in 3MGA, but also a more severe eye defect than seen in MGA-III patients. To explore whether Opa3 acts in the leucine catabolic pathway, we delivered exogenous leucine to Opa3-deficient embryos. As a comparison, leucine delivery to an MGA-I model deficient for the leucine catabolic pathway enzyme 3-methylglutaconyl-CoA hydratase causes no morphological defects, but leads to a sharp increase in leucine, and 3MGA. In contrast, leucine-treated Opa3-deficient embryos display severe brain dysmorphology but no accumulation of leucine or 3MGA. We also examined the effects on Opa3-deficient embryos of mevalonate-depletion via simvastatin treatment, and found that simvastatin causes additional brain defects in Opa3-deficient embryos. We have thus uncovered two classes of metabolic sensitivity that are specific to the brain of our zebrafish MGA-III model, indicating that zebrafish Opa3 interacts with both the leucine and mevalonate pathways.

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Program/Abstract # 317
Imaging of intestinal lipid absorption and processing in a live zebrafish
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The larval zebrafish (Danio rerio) is an ideal model of vertebrate intestinal physiology because of its rapid development and optical clarity. This system allows for the direct observation of fluorescent lipids, fusion proteins, and reporter gene constructs within the developing animal. Data from a variety of vertebrates indicates that dietary lipids are initially absorbed by intestinal enterocytes. Since energy homeostasis relies on dietary lipids, how enterocytes process lipids profoundly impacts whole animal lipid metabolism. While several studies implicate various subcellular compartments and proteins in the absorptive process, many questions remain. The highly dynamic nature of the absorptive process and the subcellular structures are difficult to image in mammals. An accurate model of intestinal physiology will reflect the combined action of both organelles as well as many cell types. We have begun the characterization of lipid uptake by zebrafish intestinal enterocytes. The ability to perform real-time in vivo lipid absorption...