cystine-knot growth factors (including VEGF, BMPs, TGFβs, and PDGFs), and is dynamically expressed during embryogenesis. We have previously shown that a Crim1 hypomorphic mouse mutant (Crim1^{KST264}) displays perinatal lethality with defects in multiple organ systems. Here we report data from a conditional mutant mouse line to produce embryos null for Crim1. Like Crim1^{KST264} mice, mice null for Crim1 displayed digit syndactyly, eye and renal defects, and exencephaly at variable penetrance. However, Crim1 null mice die by 17 dpc with severe cardiac defects, including ventricular septal defects and coronary and epicardial malformations. Moreover, some of the phenotypes resemble those of mice with defects in cystine-knot growth factors, including BMP4. Thus, we hypothesized that the phenotype of Crim1 mutants is due to aberrant TGFβ superfamily signaling, resulting in early patterning defects. Immunohistochemistry revealed reduced levels of phosphoSMAD1/5/8 protein in Crim1 mutant embryos. Furthermore, qRT-PCR showed changes in Id3 and Runx3, indicative of alterations in BMP (and TGFβ) signaling pathways. We conclude that Crim1 is essential for normal development, which may occur through modulation of BMP/TGFβ superfamily of growth factors.

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Program/Abstract # 227
The activity of cerberus-like 2 during cardiogenesis, morphological and morphogenetics studies
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Mouse *cerberus-like 2* (*cerl-2*) is a Cerberus/Dan family member that is asymmetrically expressed on the right side of the mouse node. *cerl-2* encodes for a secreted protein that binds directly to *nodal* thus inhibiting its signaling pathway. *cerl-2* KO mice display multiple laterality defects including randomization of the L/R axis. However, we have found *cerl-2*-associated cardiac defects that cannot be explained by laterality abnormalities (incomplete atrial and ventricular septation). We observe a consistent increase of ventricular muscle and to access whether this singular phenotype is independent of LR establishment we have used the transgenic mouse line *mlc1v-nLacZ24* as a correct right ventricule/OFT orientation. Based in our observations, we propose that in addition to the previously described laterality-related defects, another distinct mechanism may contribute to the spectrum of complex cardiac defects in *cerl-2* KO mice. The molecular basis of vertebrate cardiogenesis is increasingly becoming unraveled. Research in this area will be an essential step, as the targets will be the most amenable sites of intervention, both in a therapeutic sense and for the purpose of prevention. Considering the high conservation of genetic pathways regulating cardiac development in species, the study of the mouse/human orthologue genes involved in the nodal signaling pathway should bring us new data on Congenital Heart Disease (CHD) and on laterality defects.

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Program/Abstract # 228
Bves and NDRG4 modulate epicardial cell differentiation
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The epicardium differentiates to contribute cells to the coronary vasculature. In development a portion of the epicardial cells undergo EMT, delaminate, and differentiate into vascular smooth muscle (SM). We have discovered a pair of proteins, Bves and NDRG4, which biochemically interact in epicardial cells and colocalize in cells that have mesenchymal morphology. The cellular functions attributed to Bves and NDRG4 are complementary: they both affect cellular morphology, migration rate, or proliferation; all components of the overall differentiation state. Bves and NDRG4 may synergistically regulate differentiation in the epicardium. To test this, we are using an epicardial cell line that retains the ability to differentiate in culture to modify Bves and NDRG4 expression and assay for presence of differentiation markers via QRT-PCR and IF. Initial data show that overexpression of Bves or NDRG4 enhances SM marker expression. We are currently testing differentiation after co-overexpression and knockdown in the epicardial cells. To complement these studies, cryosections of an NDRG4$^{-/-}$ mouse are being investigated using IF to determine if the epicardium is intact and if the coronary vessels develop properly. Additionally, we are using a technique that involves culturing an embryonic heart to facilitate migration of the epicardium into a culture dish to investigate differentiation in the NDRG4 knockout via QRT-PCR and IF. We expect to see impaired expression of differentiation markers. Together these data will determine if Bves and NDRG4 synergistically affect differentiation in the epicardium.

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Program/Abstract # 229
Fgf3 and Fgf10 are required redundantly for neural crest migration and cardiovascular development
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Heart development requires contributions from, and interactions between, discrete cell populations including primary and secondary heart fields (SHF), cardiac neural crest (CNC), and the proepicardial organ (PEO). *Fgf3* and *Fgf10* are expressed in sites relevant to early heart development, including the hindbrain, pharyngeal endoderm, SHF and PEO, but single null mutants do not have significant heart defects. *Fgf3; Fgf10* double mutants, however, die by E11.5. These embryos lack NC-derived proximal 9th cranial ganglia and 4th pharyngeal arch arteries and arch segmentation, and exhibit pericardial edema and dilated atria suggestive of heart failure. Heart tube looping and chamber morphogenesis proceed normally, but hypoplastic ventricles and outflow tract cushions are observed with variable penetrance. To test the hypothesis that *Fgf3* and *Fgf10* are coordinately required for correct migration and/or survival of CNC cells, and for normal heart development, we assessed expression of relevant markers. Specification and early migration of NC are normal, but migration is reduced by E9.5–10.5. Expression of *Islet1* is markedly reduced in the SHF, whereas *Fgf8* and *Fgf15* are unaffected. Double mutants also show posterior pole defects, including reduced investment of epicardial cells from the PEO. Studies are underway to determine the spatiotemporal relationships between *Fgf3*, *Fgf10* and their receptors, and to determine the expression sites required for normal CNC and cardiovascular development.

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Program/Abstract # 230
Wnt signaling promotes proliferation to pattern the zebrafish craniofacial skeleton
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Formation of the craniofacial skeleton from the neural crest (NC) requires the coordinated action of multiple tissues and signaling