**Alagille Syndrome Accelerator Award**

- **Melissa Gilbert, Ph.D. (2015)**
  Research Associate
  *Children's Hospital of Philadelphia*

"Functional Implications of Thrombospondin 2 Overexpression on Liver Disease Severity in Alagille Syndrome"

Key Words: Alagille syndrome, AGS, thrombospondin, THBS2, biomarker, genetics, zebrafish, liver, jagged 1, JAG1, morpholino

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Alagille syndrome (ALGS) is an inherited disorder caused by mutations in the Notch signaling genes JAGGED1 (JAG1) and NOTCH2. ALGS primarily affects the liver, although other organs are also often disrupted. Interestingly, the liver phenotype seen in patients is highly variable, with some patients requiring transplantation while other patients experience resolution of their cholestasis. Although genetics is recognized as the disease-causing factor, with JAG1 and NOTCH2 mutations present in 96% of all ALGS patients, there has been no identified phenotype-genotype correlation between mutation type and disease progression. This inability to predict the course of the disease has severe clinical implications, with doctors often unable to provide the right timeline for therapies-often leading to liver transplantations at later stages in disease progression when children are less healthy and morbidity and mortality is increased. Our lab recently identified a single nucleotide polymorphism (SNP) through a genome-wide association study (GWAS) located upstream of the gene, THROMBOSPONDIN 2 (THBS2), that correlates with a more severe liver phenotype in patients who also have mutations in JAG1, and which we predict could act as a genetic modifier of the disease. This gene functions in the Notch signaling pathway where it is capable of inhibiting the interaction, and consequent downstream function, of JAG1 and NOTCH2. We hypothesize that increased levels of THBS2 in patients with a JAG1 mutation may further perturb Notch signaling and lead to a more severe liver phenotype in ALGS. The work here aims to study the functional implications of THBS2 overexpression in an established zebrafish model of ALGS with downregulated JAG1. We also plan to genotype patient samples for the SNP identified in our GWAS to determine if we can use this information as a biomarker to identify populations that are at risk for developing more severe liver phenotypes.

- **Hamed Jafar-Nejad, M.D. (2015)**
  Associate Professor
  *Baylor College of Medicine*

"Negative Regulation of Jagged1 (JAG1) by glocosylation: towards a mechanism-based therapy for Alagille Syndrome"

Key Words: Jagged1, glocosylation, mouse model

The overarching goal of this project is to understand the pathophysiology of bile duct defects in a congenital cholangiopathy called Alagille Syndrome (AGS) and to develop a therapeutic approach to prevent the progression of the biliary abnormalities in AGS patients. The progressive nature of bile duct paucity in many AGS patients suggests that a window of opportunity exists if an effective intervention is found and applied early. It has been known for many years that dominant mutations in the Notch pathway ligand JAG1 are responsible for ~95% of AGS cases. However, a mechanism-based therapy for this disease still does not exist. This is in part because of the lack of a representative animal model, as Jag1 heterozygous (Jag1+/-) mice on a mixed background are reported not to have any biliary phenotypes. Our preliminary data indicate that on a C57BL/6 background, Jag1+/- mice exhibit impaired bile duct development and can therefore serve as a model for the cholangiopathy of AGS. Moreover, genetic and biochemical data indicate that a glycosyltransferase called POGLUT1 (Rumi) is a dominant genetic suppressor of Jag1 haploinsufficiency in mice and suggest that POGLUT1 regulates the function of JAG1 by directly adding glucose residues to its extracellular domain. In this proposal, we will pursue two Specific Aims. In Aim 1, we will perform cell culture and biochemical experiments to determine the molecular basis for negative regulation of JAG1-induced signaling by POGLUT1. In Aim 2, which will be performed in collaboration with a company, we will determine whether decreasing the level of POGLUT1 by specific antisense oligonucleotides can improve bile duct defects in our mouse model of AGS. If accomplished, these studies will characterize a novel mechanism to regulate the function of JAG1 and can potentially establish a framework for a mechanism-based therapy for AGS.