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**Presentation Title:** Inherited *DICER1* mutations in familial pleuropulmonary blastoma

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**Introduction:** Pleuropulmonary blastoma (PPB) is a rare lung sarcoma that arises during fetal development. The majority of patients with PPB are diagnosed before 6 years of age. The early stage of PPB is characterized by cysts lined with benign epithelial cells and small numbers of subepithelial, uncommitted mesenchymal cells. In later stages the mesenchymal cells transform and become solid high-grade, multipatterned sarcomas. Sarcomatous overgrowth by the mesenchymal component dictates clinical outcome. Approximately 25% of children with PPB have a personal or family history suggestive of underlying inherited cancer susceptibility. Some children with PPB have multifocal lung tumors and/or synchronous or metachronous embryonal cancers. Family members are at increased risk for developing PPB, Wilms tumor-related neoplasms, rhabdomyosarcoma, and a number of other conditions.

**Methods:** Review of family histories for sixty-five PPB probands obtained through the International PPB Registry [www.ppbregistry.org] revealed 11 families with apparent inherited predisposition to PPB as evidenced by two or more relatives with PPB, lung cysts, cystic nephroma, and/or embryonal rhabdomyosarcoma. Blood and/or saliva specimens were collected as a source of genomic DNA. All research subjects provided written consent for molecular and family history studies as approved by the Human Research Protection Office at Washington University in St. Louis. We used Affymetrix 6.0 human SNP arrays to genotype 49 individuals from four families (14 affecteds). A total of 4,117 SNPs were selected for the analysis. Genome wide parametric linkage analysis was performed using the Genehunter v2.1r5 algorithm. Subsequent sequencing, PCR, and immunohistochemistry were performed using standard methods. DICER1 immunohistochemistry was performed with a rabbit polyclonal anti-DICER1 antibody (HPA000694, rabbit anti-human; Sigma-Aldrich, St. Louis, MO).

**Results:** Linkage analysis suggested a PPB locus on a 7 Mb interval on chromosome 14q31-32 containing 72 genes. Sequencing identified germline *DICER1* mutations in the four families included in the linkage analysis and seven additional PPB families. 10 mutations resulted in premature stop codons proximal to the functional RNase III domains. The 11th mutation resulted in a leucine to arginine change in a conserved amino acid between the two functional RNase domains. All 11 sequence defects appear to be loss-of-function mutations. RT-PCR and direct sequencing demonstrated marked reductions in levels of mutant *DICER1* transcripts compared with wild-type, suggestive of nonsense-mediated decay. Immunohistochemical analysis of DICER1 in PPB tumors from mutation carriers showed retention of staining in the mesenchymal/sarcomatous components of the tumors. In contrast, focal or segmental loss of DICER1 staining was seen specifically in the benign epithelial component of the tumors.

**Conclusions:** The discovery of germline *DICER1* mutations in affected members of PPB kindreds, coupled with absence of DICER1 protein in tumors, suggests DICER1 loss is responsible for familial PPB. DICER1 is responsible for generating active miRNAs (and siRNAs). Our immunohistochemical studies, in which we show loss of DICER1 in the epithelial component of tumors but retention of DICER1 expression in the transformed mesenchymal cells, provide evidence that DICER1 loss promotes malignant transformation through a non-cell autonomous mechanism. In the mouse, loss of DICER1 in the epithelium of the developing lung alters epithelial-mesenchymal signaling, resulting in a lung phenotype that mimics early PPB (Harris et al.; PNAS 2006;103:2208-13). Our study of hereditary PPB suggests the primary defect does not occur in the mesenchymal cell (as was long suspected), but rather in the epithelial cell, and it extends the findings in mice to human tumorigenesis. We hypothesize that loss of DICER1 in lung epithelium promotes both cyst formation and tumor initiation through dysregulation of developmental genes that are normally regulated by miRNAs. Further study of PPB families and the tumors that arise in *DICER1* mutation carriers represent a unique opportunity to learn about the cellular processes in the borderland between development and neoplasia and to study how tissue-specific loss of DICER1 (and the miRNAs it regulates) manifests in human disease.