



Pathology Resequencing Laboratory
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DICER1 Mutation Testing Information Sheet

DICER1 (OMIM: *606241) mutation associated diseases: Pleuropulmonary blastoma (OMIM: #601200); pleuropulmonary blastoma family cancer predisposition syndrome; cystic nephroma; ovarian Sertoli-Leydig tumor or sex cord-stromal tumors; embryonal rhabdomyosarcoma

Clinical Features:

Pleuropulmonary blastoma (PPB) is a tumor that arises during lung development or shortly after birth. The tumor has three pathologic forms. The earliest form appears as a multilocular cyst within the lung parenchyma (Type I PPB).^{1,2} Cyst(s) are typically lined by alveolar-type epithelium. Cyst walls contain variable numbers of mesenchymal cells which are either uncommitted or show skeletal muscle or cartilaginous differentiation. In later stages of tumorigenesis, the mesenchymal cells expand and overgrow the epithelial cysts forming an overtly malignant cystic and solid (Type II) or purely solid sarcoma (Type III).^{1,2} These children are older than those with Type I PPB; the median ages at diagnosis for Type II and Type III PPB are 36 and 42 months respectively. A child with Type II or III PPB typically presents with weight loss, fever, shortness of breath and a large lung mass. These pathologic types of PPB reflect the observed biological progression within individual patients and in age-segregated subgroups.^{1,2} Children presenting with Type III PPB represent approximately one-third of all PPBs.

Pathologic type is the only known statistically significant predictor of outcome and prognosis.³ Type I is used to stratify patients into different therapeutic groups. Type I PPB is optimally treated with complete surgical removal and often adjuvant chemotherapy. With chemotherapy, the overall 5-year survival for this subgroup is over 90%.⁴ Children with Types II and III PPB are subject to surgery as well as an aggressive chemotherapy regimen, however, the 5-year survival rates are only 60% and 45%, respectively. These survival statistics suggest that screening methods which improve our ability to identify PPB in its early stages may dramatically improve outcome.

Genetics:

Early observations in children with PPB showed that the disease was commonly multifocal and could affect relatives.⁵ Approximately 25% of children with PPB have a personal or family history of other childhood cancers. Cancers and other conditions described in these families include other PPBs, lung cysts, embryonal rhabdomyosarcoma, Wilms tumor, cystic nephroma, intraocular medulloepithelioma, nasal chondromesenchymal hamartoma, medulloblastoma, glioblastoma, gonadal germ cell and stromal tumors, thyroid cancers and hyperplasias.

DICER1 is currently the only gene identified to be associated with this group of cancers/conditions.⁶ *DICER1* mutations are inherited in an autosomal dominant fashion. Approximately 70% of all children with PPB harbor germline mutations in *DICER1*. The majority of these mutations are base substitutions creating premature stop codons or small insertions or deletions causing frameshifts and premature stop codons which prevent the *DICER1* protein from functioning properly. A small number of missense mutations have been identified and are predicted to be deleterious and correlate with presence of disease within a family. The prevalence of *DICER1* mutations in individuals presenting only with the other associated conditions and without PPB in the family is unknown. The penetrance for diseases associated with *DICER1* mutations is not known. Very few families have more than one child with pleuropulmonary blastoma. Clinical research is being performed to determine disease risk in individuals carrying *DICER1* mutations.

References:

1. Hill DA, et al. Am J Surg Pathol 2008; 32(2):282-295.
2. Priest JR, et al. Cancer 1997; 80(1):147-161.
3. Hill DA, et al. Lab Invest 2006; 86:212-227.
4. Priest JR, et al. J Clin Oncol 2006; 24(27):4492-4498.
5. Priest JR, et al. J Pediatr 1996; 128(2):220-224.
6. Hill DA, et al. Science 21 August 2009:Vol. 325. no. 5943, p. 965

Reasons for Referral:

1. Confirmation of clinical diagnosis in symptomatic patients
2. Risk assessment of asymptomatic or presymptomatic family members of a proband with PPB or a related condition
3. Genetic counseling and recurrence risk calculation
4. Clinical laboratory confirmation of a research laboratory result

Testing Methodology:

Genomic DNA is extracted from whole blood samples. Polymerase chain reaction is performed using primers for all 26 coding exons including intron-exon junctions. DNA is sequenced using conventional dideoxy sequencing on an Applied Biosystems analyzer. The DNA sequence is assembled and compared to the published genomic reference sequences (RefSeqNM_030621) using the Sequencher program. Sequence variants are assessed for effect on protein product. When possible, correlation with other affected family member's sequence is performed. If no sequence variants consistent with disease causation are identified, the sample can be referred for deletion testing. We will contact the ordering physician to determine if deletion testing is desired. A quantitative PCR assay is used for analyzing for deletions of all or part of individual coding exons 2-26. Each amplicon signal is compared to known, validated two-copy internal control exons.

Test Sensitivity:

Based on our preliminary research findings, approximately 70% of individuals with a clinical diagnosis of PPB are expected to harbor a mutation in the coding region of *DICER1*. Based on the performance of our assay we anticipate >95% rate of detection of coding region mutations or exon deletions. Our assay will not identify promoter mutations, deep intronic mutations and/or methylation changes that may affect *DICER1* expression.

Turnaround Time:

DICER1 sequencing results can be expected within 6 weeks from receipt of specimen.

Specimen Requirements:

- Blood*: A single tube with 5-10 mL whole blood in EDTA (purple top) or ACD (yellow top).
- Other Specimens*: Contact us for specific inquiries and specimen requests.

Required Forms:

- DICER1* Testing Sample Submission (Requisition) Form – complete all pages
- Payment Options Form or Institutional Billing Instructions
- Please submit relevant clinical information (pathology report, operative report, pedigree) with specimens. Alternatively, clinical information can be sent directly to the laboratory by secure e-mail to dashill@cnmc.org or by fax to (202) 476-4030.

Shipping/Handling:

Specimen collection kits will be provided upon request.
Ship blood at ambient temperature (or using a cool pack in hot weather).
Please ship blood overnight priority delivery on Monday through Thursday.
The laboratory is not open to receive specimens on Saturdays.
If shipping on Friday please check for priority Monday morning delivery or call the laboratory to make special arrangements.
If shipping samples from overseas, please contact the laboratory.

Ship blood to:

Pathology Resequencing Laboratory
Attn: D. Ashley Hill, M.D.
Children’s National Medical Center
Department of Pathology, Rm 1620
111 Michigan Ave. NW
Washington, DC 20010 U.S.A.
(202) 476-2051 phone
(202) 476-4030 fax

Please notify the laboratory by e-mail at dashill@cnmc.org regarding specimen delivery, including courier and tracking number.

Potential ICD-9 codes: 162.9 (malignant neoplasm of bronchus or lung, unspecified)
748.4 (congenital lung cyst)

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Children’s National Medical Center Tax ID: 530196508

CPT codes:

DICER1 mutation detection in a new patient
83891 x 1
83898 x 62
83894 x 62
83904 x 62
83912 x 1

DICER1 mutation carrier testing with known mutation site (documentation must be provided if previous testing not done in CNMC laboratory).
83891 x 1
83898 x 2
83894 x 2
83904 x 2
83912 x 1