Cancer-associated Mutations in Congenital Pulmonary Malformations: A Prospective Cohort

To the Editor:

Cancer-associated mutations are identified in some congenital pulmonary malformations (CPMs), including mutations of the V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene in particular (1, 2). They were described in cystic malformations, with or without mucinous islets (1, 2). The theoretical risk of a link between CPMs and cancer risk is fueling the debate about whether preventive surgery should be performed for asymptomatic CPM.

We used the large French prospective population-based MALFPULM cohort to evaluate the actual prevalence of cancer-associated mutations in CPMs and to correlate CPM phenotype with the presence of at least one oncogenic mutation. This cohort has been described in detail elsewhere (3, 4). Briefly, all pregnant women whose fetus had a prenatal diagnosis of CPM were invited to participate in the study, whatever the ultrasonic phenotype of the CPM: cystic, purely hyperechoic, or mixed. This study received institutional review board approval (Comité de Protection des Personnes Ile-de-France IV, U.S. Department of Health and Human Services Agreement no. 00003835). Follow-up of this cohort was performed up to 2 years of age and had no impact on the decision as to whether or not to perform elective surgery. When compared with children with elective surgery, nonoperated children had fewer cystic malformations but as many malformations with systemic vascularization or emphysematous images. Postnatal thoracic computed tomography description and final histological diagnosis (5) were prospectively collected in a online database. Tumor DNA was extracted from macrodissected tissue samples from the area of the malformation. The 22 gene Ion AmpliSeq Colon and Lung Cancer Research Panel v2 (Life Technologies–Thermo Fisher Scientific) was used. Mutations in DNA were detected at allele frequencies as low as 0.001 for insertions or deletions >2 bp and 0.003 for single-nucleotide variants (6). For CPMs with mutation, tissue samples from apparently normal lung parenchyma, collected at some distance from the malformation sample, were also tested, when available. A blind histological review of slides from CPMs with mutation, together with randomly selected slides from 15 tissues without mutation, was performed using a standardized grid that was created by the review panel of four pathologists from three centers.

Univariate analysis compared the following parameters between malformations with mutations and those without mutations: pre- and postnatal imaging appearance of the malformation; maximum value during pregnancy of the ratio of CPM volume to head circumference (CVRmax); severe fetal compression; and neonatal respiratory distress (3, 4). χ^2 tests, Fisher's exact tests, *t* tests, and univariate logistic regression were used.

Of the 426 live-born infants in the MALFPULM cohort, 285 underwent surgery before the age of 2 years, and 195 of them were genotyped. The main reason for the absence of genomic analysis was the omission of the additional consent collection. The characteristics of CPMs sent for analysis did not differ from the ones not sent. A *KRAS* mutation was identified in 17 cases and an fibroblast growth factor receptor 2 (*FGFR2*) mutation in one case (Table 1). Seven CPMs with mutation had a concomitant analysis of histologically healthy tissue. In four of them, the same mutation was identified as in the area of the malformation, with similar or lower allelic ratios.

CPMs with mutation demonstrated higher prenatal CVRmax values (odds ratio, 1.08; 95% confidence interval, 1.02–1.14 for every 0.1 cm^2 increase in CVRmax; $P = 0.007$), higher rate of prenatal compressive complications ($P = 0.037$), higher rate of neonatal respiratory distress ($P = 0.024$), higher rate of surgery in the first week of life ($P < 0.001$), and higher rate of surgery because of symptoms ($P < 0.001$) (Table 2). All CPMs with mutation were cystic in appearance on postnatal imaging ($P = 0.001$), with no difference in postnatal mean diameter of the largest cyst between CPMs with or without mutation. In 3 of the 18 CPMs with mutation, the cysts were not detected on prenatal imaging. The histological diagnosis was congenital pulmonary airway malformation (CPAM) for all CPMs with mutations ($P = 0.01$). Of the 66 CPMs with postnatal systemic vascularization, including 17 who were also cystic, none had a mutation ($P = 0.002$). Microscopically, a mucinous component was identified only in tissues with mutation ($P < 0.005$). However, 56% of the mutated tissues had no identifiable mucinous component on the slides reviewed. Inflammation was noted in more than half the tissues, with no association with the presence of mutations. Microscopy findings were consistent with the diagnosis of type 2 CPAM in five cases with mutations.

In our prospective cohort, we demonstrate that mutations of cancer-associated genes are identified only in CPMs with a cystic component on postnatal imaging, with a prevalence of 15% within these cystic CPMs. All cystic CPMs are at risk of mutation, regardless of cyst size, type of CPAM, or identifiable mucinous epithelial cells by microscopy. This is concordant with the previous description of *KRAS* mutations away from mucinous islets of type 1 CPAMs or in type 2 CPAMs (1). Among the 22 genes tested on our panel, only KRAS and FGFR2 were identified with mutations. The *FGFR2* mutation in one case was the first to be described in a CPM. As one-third of tissues with mutation were obtained from neonates, the results suggest that these mutations are prenatally acquired,

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Author Contributions: C.D. and B.K. conceptualized and designed the study, supervised the analysis, drafted the initial manuscript, and reviewed and revised the manuscript. M.R. designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. S.G. and H.B. carried out the genomic analyses, supervised genomic analysis, and reviewed and revised the manuscript. L.G.-R. supervised tissue sample collection, reviewed tissue slides, contributed to data interpretation, and critically reviewed the manuscript for important intellectual content. O.A., A.B., F.H., and N.K.-D. coordinated and supervised data collection, contributed to data interpretation, and critically reviewed the manuscript for important intellectual content. All the authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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This letter has a related editorial.

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Table 1. Description of the 18 Cases with Mutation

Case Number	Age at Surgery (*d*)	Mutation in Malformed Tissue	Allelic Ratio (%)	Mutation in Surrounding Healthy Tissue	Allelic Ratio (%)	Prenatal Imaging	Birth Weight (*kg*)	Neonatal Respiratory Distress*	Largest Cyst Diameter on Postnatal CT (*mm*)	Mucinous Component
1	641	*KRAS* p.Gly12Asp c.35G>A	13.2	KRAS p.Gly12Asp c.35G>A	15	Hyperechoic	2.8	No	9	No
2	1	*KRAS* p.Gly12Asp c.35G>A	13	*KRAS* p.Gly12Asp c.35G>A	4	Cystic	3.3	Yes	41	No
3	0	*KRAS* p.Gly12Asp c.35G>A	23	NA		Cystic	3.5	Yes	45	Yes
4	217	*KRAS* p.Gly12Asp c.35G>A	10	None	—	Cystic	3.7	No	12	Yes
5	320	*KRAS* p.Gly12Val c.35G>T	4	NA		Hyperechoic	3	Yes	7	No
6	192	*KRAS* p.Gly12Asp c.35G>A	11	NA		Cystic	3.5	No	33	No
7	211	*KRAS* p.Gly12Asp c.35G>A	20	NA		Cystic	3.2	No	34	Yes
8	410	*KRAS* p.Gly12Val c.35G>T	3	NA		Cystic	4.1	No	11	No
9	783	*KRAS* p.Gly12Asp c.35G>A	12	NA		Cystic	3.5	No	21	Yes
10	364	*KRAS* p.Gly12Val c.35G>T	20	NA		Cystic	3.4	Yes	40	No
11	118	*KRAS* p.Gly12Asp c.35G>A	19.4	None	—	Cystic	3.1	No	17	No
12	4	*KRAS* p.Gly12Asp c.35G>A	18.7	KRAS p.Gly12Asp c.35G>A	0.7	Cystic	2.6	Yes	20	Yes
13	13	*KRAS* p.Gly12Asp c.35G>A	33	KRAS p.Gly12Asp c.35G>A	9	Cystic	3.3	No	35	Yes
14	391	*KRAS* p.Gly12Asp c.35G>A	4.2	NA		Cystic	3	No	26	Yes
15	190	*KRAS* p.Gly12Asp c.35G>A	7.9	None	—	Cystic	3.8	No	33	No
16	234	*KRAS* p.Gly12Arg	27	NA		Cystic	3.6	No	24	No
17	2	*KRAS* p.Gly12Cys	17	NA		Cystic	2.9	Yes	53	Yes
18	0	FGFR2 p.Cys382Arg c.1144T>C	34	NA		Hyperechoic	3.2	Yes	NA	No

Definition of abbreviations: CT = computed tomography; NA = not applicable.
*Neonatal respiratory distress was defined by having at least one of the following criteria: polypnea > 60/min or signs of retraction; need for oxygen therapy, noninvasive ventilatory support, or invasive ventilatory support; need for surgical congenital pulmonary malformation removal before the age of 7 days (3).

Table 2. Characteristics of Patients and Lesions by Mutation Status

	No Mutation	With Mutation	*P* Value
Prenatal data			
Prenatal US			
Hyperechogenic	61 (34.5)	3 (16.7)	0.126
Cystic image	116 (65.5)	15 (83.3)	
CVRmax, cm^2	0.67 ± 0.59	1.24 ± 1.35	0.004
Compression*			
No	166 (93.8)	14 (77.8)	0.037
Yes	11 (6.2)	4 (22.2)	
Neonatal and postnatal data			
Birth weight, kg	3.3 ± 0.5	3.3 ± 0.4	0.830
Prematurity			
No	164 (92.7)	15 (83.3)	0.172
Yes	13 (7.3)	3 (16.7)	
Neonatal respiratory distress†			
No	149 (84.2)	11 (61.1)	0.024
Yes	28 (15.8)	7 (38.9)	
Cystic image			
No	72 (40.7)	0	0.001
Yes	105 (59.3)	18 (100)	
Cyst diameter, mm	22.1 ± 14.8	27.1 ± 13.6	0.200
Cyst diameter > 20 mm			
No	56 (59.6)	6 (35.2)	0.064
Yes	38 (40.4)	11 (64.7)	
Systemic vascularization			
No	110 (62.1)	17 (100)	0.002
Yes	66 (37.3)	0	
Median age at surgery, d	265	202	<0.001
Age at surgery			
⩽7 d	2 (1.1)	5 (27.8)	<0.001
>7 d	175 (98.9)	13 (72.2)	
Surgery indication			
Symptomatic	21 (12.0)	8 (44.4)	<0.001
Elective	154 (88.0)	10 (55.6)	
Histology by local pathologist			
CPAM	81 (45.8)	18 (100)	0.010
Sequestration	55 (31.1)	0	
Bronchial atresia	3 (1.7)	0	
Congenital lobar emphysema	5 (2.8)	0	
Bronchogenic cyst	9 (5.1)	0	
CPAM + sequestration (hybrid)	11 (6.2)	0	
Other	6 (3.4)	0	
Pathological data with histological review‡			
Cuboidal epithelial cells	3 (20)	8 (44)	0.174
Columnar ciliated epithelial cells	10 (71)	17 (94)	0.075
Mucinous epithelial cells	0	8 (44)	0.004
Cartilaginous nodules	3 (20)	0	0.047
Chronic inflammation	9 (60)	10 (56)	0.797

Definition of abbreviations: CPAM = congenital pulmonary airway malformation; CVRmax = maximum value during pregnancy of the ratio of CPM volume to head circumference; US = ultrasound.
Data are presented as *n* (%) or mean ± SD unless otherwise noted.
*Presence of hydrops, hydrothorax, ascites, or hydramnios during pregnancy.
†Neonatal respiratory distress was defined by having at least one of the following criteria: polypnea > 60/min or signs of retraction; need for oxygen therapy, noninvasive ventilatory support, or invasive ventilatory support; need for surgical congenital pulmonary malformation removal before the age of 7 days (3).
‡*n* = 15 tissues without mutation for histological review.

consistent with the description of neonatal mucinous adenocarcinoma associated with CPAM and *KRAS* mutations (7).

The fact that mutations are associated with larger and more symptomatic CPMs raises questions about their role in the pathophysiology of CPMs. KRAS gain of function has been shown to result in an increase in the branching program, with a cystic airway branch phenotype, and the suppression of alveolar differentiation (8). Similarly, the p.Cys382Arg *FGFR2* mutation is known to result in receptor activation, and high FGF10/FGFR2 activity in embryonic lungs has been shown to lead to cyst formation in both rodents (9) and human fetal explants (10).

Cancers other than mucinous adenocarcinomas have been associated with CPMs, including rhabdomyosarcoma or pleuropulmonary blastoma. However, these lesions are now believed

to differ fundamentally from CPMs because of the lack of prenatal diagnosis (11) and a different genetic and molecular background (12). Thus, the discussion of tumor risk of prenatally diagnosed malformations remains focused on mucinous adenocarcinomas, which are themselves mostly associated with a KRAS mutation.

The detection of *KRAS* mutations in apparently healthy areas distant from the cystic area raises questions about the need for systematic searches for these mutations in surgical specimens and for long-term monitoring in children with mutations. However, to our knowledge, there are no published cases of mucinous adenocarcinoma occurring during follow-up after surgery for CPM in the absence of an initial cancerous lesion. Thus, although our findings support specific management of cystic CPMs, either preventive removal or long-term follow-up, they do not support systematic searches for cancer-associated mutations in surgical specimens or particular follow-up for children undergoing surgery with a mutation that would have been identified in the malformative tissue. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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References

1. Hermelijn SM, Wolf JL, Dorine den Toom T, Wijnen RMH, Rottier RJ, Schnater JM, *et al.* Early KRAS oncogenic driver mutations in nonmucinous tissue of congenital pulmonary airway malformations as an indicator of potential malignant behavior. *Hum Pathol* 2020;103:95–106.

2. Lantuejoul S, Nicholson AG, Sartori G, Piolat C, Danel C, Brabencova E, *et al.* Mucinous cells in type 1 pulmonary congenital cystic adenomatoid malformation as mucinous bronchioloalveolar carcinoma precursors. *Am J Surg Pathol* 2007;31:961–969.
3. Delacourt C, Bertille N, Salomon LJ, Rahshenas M, Benachi A, Bonnard A, *et al.*; for the MALFPULM study group. Predicting the risk of respiratory distress in newborns with congenital pulmonary malformations. *Eur Respir J* 2022;59:2100949.
4. Delacourt C, Bertille N, Salomon LJ, Benachi A, Henry E, Massardier J, *et al.*; Prenatal MALFPULM Study Group. Prenatal natural history of congenital pulmonary malformations: MALFPULM population-based cohort study. *Ultrasound Obstet Gynecol* 2019;54:381–388.
5. Langston C. New concepts in the pathology of congenital lung malformations. *Semin Pediatr Surg* 2003;12:17–37.
6. Pécuchet N, Rozenholc Y, Zonta E, Pietrasz D, Didelot A, Combe P, *et al.* Analysis of base-position error rate of next-generation sequencing to detect tumor mutations in circulating DNA. *Clin Chem* 2016;62: 1492–1503.
7. Muntean A, Banias LE, Ade-Ajayi N, Patel SB, McKinney O, Davenport M. Neonatal congenital pulmonary airway malformation associated with mucinous adenocarcinoma and KRAS mutations. *J Pediatr Surg* 2022; 57:520–526.
8. Chang DR, Martinez Alanis D, Miller RK, Ji H, Akiyama H, McCrea PD, *et al.* Lung epithelial branching program antagonizes alveolar differentiation. *Proc Natl Acad Sci USA* 2013;110:18042–18051.
9. Gonzaga S, Henriques-Coelho T, Davey M, Zoltick PW, Leite-Moreira AF, Correia-Pinto J, *et al.* Cystic adenomatoid malformations are induced by localized FGF10 overexpression in fetal rat lung. *Am J Respir Cell Mol Biol* 2008;39:346–355.
10. Danopoulos S, Thornton ME, Grubbs BH, Frey MR, Warburton D, Bellusci S, *et al.* Discordant roles for FGF ligands in lung branching morphogenesis between human and mouse. *J Pathol* 2019;247: 254–265.
11. Kunisaki SM, Lal DR, Saito JM, Fallat ME, St Peter SD, Fox ZD, *et al.*; Midwest Pediatric Surgery Consortium. Pleuropulmonary blastoma in pediatric lung lesions. *Pediatrics* 2021;147:e2020028357.
12. Dehner LP, Messinger YH, Schultz KA, Williams GM, Wikenheiser-Brokamp K, Hill DA. Pleuropulmonary blastoma: evolution of an entity as an entry into a familial tumor predisposition syndrome. *Pediatr Dev Pathol* 2015;18:504–511.

Human Leukocyte Antigen Alleles Associated with Interstitial Lung Disease in North Americans with Idiopathic Inflammatory Myopathy

To the Editor:

Idiopathic inflammatory myopathies (IIM) are rare systemic autoimmune disorders that result from complex genetic and environmental interactions. IIMs are largely characterized by muscle inflammation, but extramuscular manifestations are common (1). Interstitial lung disease (ILD) is a frequent and potentially fatal extramuscular manifestation in IIM subtypes such as dermatomyositis and antisynthetase syndrome. There is significant heterogeneity among ILD subtypes with variable degrees of risk attributable to genetic variants, environmental exposures, and systemic disorders. Common genetic polymorphisms associated with idiopathic pulmonary fibrosis (2) and other ILDs are not associated with IIM-ILD (3). Human leukocyte antigen (HLA) class I and II haplotypes carry the strongest known genetic associations with IIM, although they are not a major determinant of IIM (1). Prior studies of HLA alleles in IIM-ILD have been small and conducted primarily in subjects of European and Asian ancestry (4), and thus, it is unclear whether HLA alleles contribute to IIM ILD risk, especially in people with African ancestry. Expanding on a previously published abstract (5), we tested whether HLA polymorphisms were associated with ILD in two diverse independent cohorts of North Americans with IIM.

Methods

Our study populations were the NIH (National Institutes of Health) (discovery) cohort and the JH (Johns Hopkins) (replication) cohort. The NIH cohort included self-reported non-Hispanic European Americans and African Americans, with IIM identified from five referral centers between 1983 and 2002. The Johns Hopkins cohort included self-reported non-Hispanic European Americans and African Americans with IIM recruited from the Johns Hopkins Myositis Center between 2006 and 2018. Both cohorts used the same IIM diagnostic criteria, excluding those with inherited, metabolic, or infectious myopathies and other causes of muscle disease. All patients provided informed written consent and were enrolled in investigational review board-approved clinical protocols at National Institute of Environmental Health Sciences, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Johns Hopkins University (00,055,370), and the University of Pennsylvania (832,352).

HLA-type data for the NIH cohort were on the basis of low-resolution HLA class I and II genotyping. For the Johns Hopkins cohort, genome-wide genotype data were obtained with the Infinium ImmunoArray platform (Illumina, Feinstein Institute). After quality control filters, imputed data for European American and African American samples were obtained with Minimac4 while using 1000 Genomes Project Phase 3 European populations and CAAPA (Consortium on Asthma among African-Ancestry Populations in the Americas African American Panel for hg19), respectively, as reference. Subsequently, HLA-type data were imputed with SNP2HLA (6) while using the 1000 Genomes Project HLA data as a reference. The HLA typing strategy for the cohorts differed on the basis of the prevailing technology available at the time of genotyping.

In analyses stratified by race, we used logistic regression models to test for the association between ILD and each HLA subtype with an allele frequency greater than 5% while including sex as a covariate in all models and the top genetic principal component in the JH models. Subsequently, a meta-analysis was performed with METASOFT to obtain overall associations. Adjusted *P* values were obtained via a Benjamini-Hochberg correction to adjust for the number of

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