ARTICLE

Genetics etiologies and genotype phenotype correlations in a cohort of individuals with central conducting lymphatic anomaly

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Central conducting lymphatic anomaly (CCLA) is a heterogenous disorder caused by disruption of central lymphatic flow that may result in dilation or leakage of central lymphatic channels. There is also a paucity of known genetic diagnoses associated with CCLA. We hypothesized that specific genetic syndromes would have distinct lymphatic patterns and this would allow us to more precisely define CCLA. As a first step toward "precision lymphology", we defined the genetic conditions associated with CCLA by performing a retrospective cohort study. Individuals receiving care through the Jill and Mark Fishman Center for Lymphatic Disorders at the Children's Hospital of Philadelphia between 2016 and 2019 were included if they had a lymphangiogram and clinical genetic testing performed and consented to a clinical registry. In our cohort of 115 participants, 26% received a molecular diagnosis from standard genetic evaluation. The most common genetic etiologies were germline and mosaic RASopathies, chromosomal abnormalities including Trisomy 21 and 22q11.2 deletion syndrome, and *PIEZO1*-related lymphatic dysplasia. Next, we analyzed the dynamic contrast magnetic resonance lymphangiograms and found that individuals with germline and mosaic RASopathies, mosaic KRASopathies, *PIEZO1*-related lymphatic dysplasia, and Trisomy 21 had distinct central lymphatic flow phenotypes. Our research expands the genetic conditions associated with CCLA and genotype-lymphatic phenotype correlations. Future descriptions of CCLA should include both genotype (if known) and phenotype to provide more information about disease (gene-CCLA). This should be considered for updated classifications of CCLA by the International Society of Vascular Anomalies.

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INTRODUCTION

The lymphatic system is primarily responsible for body fluid homeostasis and the transport of dietary lipids. Complex lymphatic anomalies (CLAs) refer to a group of complex malformations of the lymphatic system (reviewed in [1, 2]). Generalized lymphatic anomaly (GLA) refers to multiple lymphatic malformations, often with localized malformations in bones or organs such as the spleen. Kaposiform lymphangiomatosis is a subset of GLA with additional features of coagulopathy, mediastinal involvement or thickening, and focal areas of spindle-shaped cells on biopsy. The complex lymphatic anomaly we will focus on in this article is called central conducting lymphatic anomaly (CCLA) or channel-type lymphatic malformation.

Central lymphatic flow involves the cisterna chyli (CC), thoracic duct (TD), and its tributaries. Central conducting lymphatic anomaly (CCLA) has been characterized as disruption of this system, resulting in dilated channels, dysmotility, or obstruction of central lymphatics. The resulting abnormal lymphatic drainage may manifest as a host of symptoms (including but not limited to) chylothorax, chylous ascites, protein-losing enteropathy, other effusions, or lymphedema [3]. The International Society for the Study of Vascular Anomalies (ISSVA) has classified CCLA as a channel-type lymphatic type malformation caused by lymphatic vessel dysfunction [4]. However, CCLA is a heterogenous diagnosis limiting proper counseling about outcomes and management decisions.

Non-contrast T2-weighted MR lymphangiography (T2MRL) and dynamic contrast MR lymphangiography (DCMRL) have allowed for characterization of the central lymphatic system [5–8]. Biko et al reported central lymphatic conduction abnormalities in individuals with a clinical or molecular diagnosis of RASopathy, the most common of which were thoracic duct anomalies, retrograde intercostal perfusion, and pulmonary lymphatic perfusion [7]. However, genotype phenotype associations have not been evaluated for other genetic syndromes associated with CCLA.

A limiting factor in this process is the lack of information about the genetic etiologies and syndromes associated with CCLA. CCLA has been associated with pathogenic or likely pathogenic variants in *ARAF*, *EPHB4*, *JAG1*, *SOS1*, and *MDFIC* [9–13]. Lymphatic anomalies have been reported in the literature in association

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with other syndromes. Most notably, pathogenic variants in the RAS/MAPK pathway cause lymphedema and lymphangiectasia in Noonan syndrome, Costello syndrome, and cardiofaciocutaneous syndrome [14, 15]. However, there has not been a previous evaluation to identify the type and incidence of genetic diagnoses in a cohort of individuals with CCLA.

We hypothesized that other genetic syndromes would have distinct lymphatic patterns and this would allow us to more precisely define CCLA. Consequently, we performed a retrospective cohort study of individuals evaluated in our Lymphatic Center from 2016 to 2019. First, we established the genotypes associated with CCLA and show that a causative molecular etiology is identified in about one quarter of cases. Next, we used the genotypes and lymphangiograms to discover novel patterns of conduction associated with known genetic syndromes.

MATERIALS AND METHODS

Participants were consented into an institutional review board approved registry and biorepository study. We conducted a retrospective analysis of 412 individuals in our registry who received care through the Jill and Mark Fishman Center for Lymphatic Disorders at the Children's Hospital of Philadelphia between January 1, 2016 and December 31, 2019. Inclusion criteria included individuals receiving a lymphangiogram at our center, with all but two having DCMRL (#24, #50) and genetic testing or genetic diagnosis. Individuals were excluded if they did not have either evaluation. Data collection included demographics, clinical presentation, genetic results, and lymphatic imaging results. Of note, individuals 1, 2, 4, 5, 6, and 7 were previously reported[7]. Individual 7, 8, 10, 16, and 69 were previously reported [10]. Individual 38 was reported [16]. Individual 49 was reported [11].

Lymphatic imaging

Clinical non-contrast T2-weighted MR lymphangiogram, dynamic contrast MR lymphangiogram, and conventional lymphangiography were performed as previously described [7]. T2-weighted lymphatic imaging was evaluated for the presence of pleural effusion, ascites, pericardial effusion, and increased T2 signal in the retroperitoneum, body wall, mesentery, mediastinum, and supraclavicular regions. DCMRL was evaluated for abnormal lymphatic perfusion into the lung, intercostal space, mediastinum, neck/supraclavicular region, mesentery, axilla, and the presence of dermal backflow, which was previously defined as retrograde movement of contrast from the inguinal lymph nodes into the subcutaneous tissues of the upper thighs and perineum [7].

Genetic analysis and classification

Medical records were abstracted for clinical genetic testing either at our institution or at a referring institution. Details of the tests, including the type of test and specific findings, were also included. Cases were classified as primary or acquired. Cases were classified as primary if the lymphatic issues were thought to be a primary malformation based on medical history. Primary cases were further categorized as confirmed molecular etiology, suspected molecular etiology, or unknown molecular etiology. Cases were classified as confirmed etiology when the existing literature has demonstrated that the gene contributes to the pathogenesis of lymphatic disease, when lymphatic disease was reported as a characteristic of the syndrome, or lymphatic symptoms such as non-immune fetal hydrops, chylothorax, or chylous ascites were described without previous imaging of the central lymphatics. Individual 27 was included in the confirmed group due to biallelic VUSes and similar red blood cell phenotype to previously reported patients with *PIEZO1*-related generalized lymphatic dysplasia [17]. Cases were classified as suspected etiology when a variant of uncertain significance was found in a gene that is known to contribute to lymphatic dysfunction or there was no other explanation after comprehensive testing (individual 38). Cases were classified as unknown etiology when no pathogenic variants were identified or when the gene/copy number variant identified has not been reported in the literature to contribute to lymphatic dysfunction. Cases were classified as acquired when the patient presented with lymphatic symptoms after a surgical intervention, most often cardiac surgery. Acquired cases were excluded.

RESULTS

Patient population

During the study period, 412 individuals were identified who received care through the Jill and Mark Fishman Center for Lymphatic Disorders at the Children's Hospital of Philadelphia between 2016 and 2019. Of those, 118 out of 412 individuals (29%) received clinical genetic testing at either our institution or an outside institution. One individual did not undergo lymphatic imaging. Two individuals had normal lymphatic imaging. Sixty-eight out of 118 individuals had a reported variant on testing. Thirteen cases were classified as acquired and excluded. Therefore, 55 individuals were included in the final analysis. A flowchart showing the inclusion and exclusion of individuals and subsequent categorization is shown in Fig. 1.

Demographic data and presenting features of the 55 individuals are summarized in Supplementary Table 1. Slightly more than half of the cohort was male (56%). Of the 55 individuals, the mean age

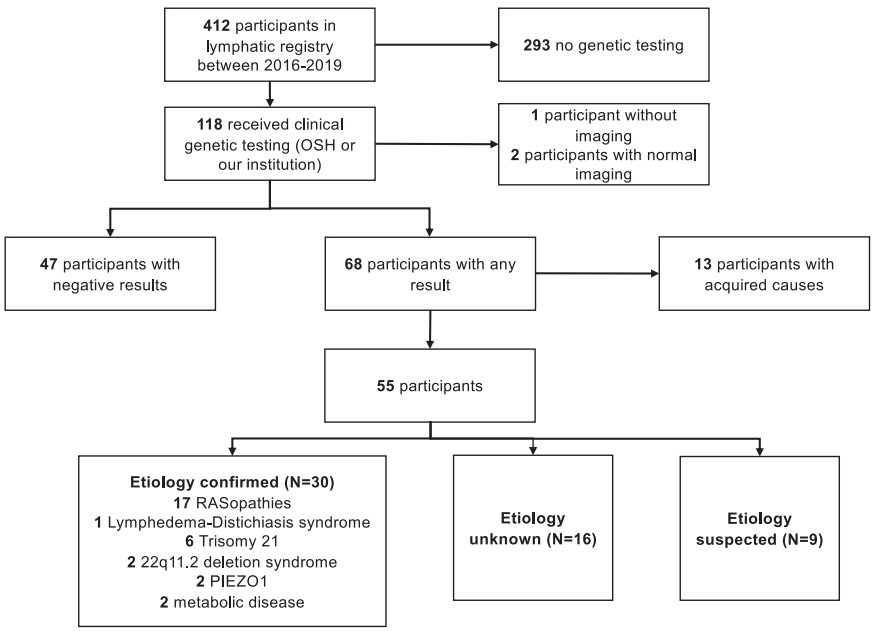

Fig. 1 CONSORT diagram. Flowchart shows the inclusion and exclusion of the study participants and subsequent categorization.

Table 1. Genetic testing results of individuals in the confirmed etiology category.

Patient ID	Category	Variant	Interpretation
1	RASopathy	NM_002834.5 *PTPN11* (c.1510A>G; p.Met504Val)	Pathogenic: Noonan syndrome
2	RASopathy	NM_002834.5 *PTPN11* (c.236A>G; p.Gln79Arg)	Pathogenic: Noonan syndrome
3	RASopathy	NM_005343.4 *HRAS* (c.34G>A; p.Gly12Ser)	Pathogenic: Costello syndrome
4	RASopathy	NM_001354689.3 *RAF1* (c.433A>C; p.Thr145Pro) *de novo*	Likely pathogenic: Noonan syndrome
5	RASopathy	NM_001256821.1 *RIT1* (c.280G>A; p.Ala94Thr)	Pathogenic: Noonan syndrome
6	RASopathy	NM_002834.5 *PTPN11* (c.1510A>G; p.Met504Val)	Pathogenic: Noonan syndrome
7	RASopathy	NM_002834.5 *PTPN11* (c.1510A>G; p.Met504Val)	Pathogenic: Noonan syndrome
8	RASopathy	NM_005633.3 *SOS1* (c.2536G>A; p.Glu846Lys)	Pathogenic: Noonan syndrome
9	Mosaic KRASopathy	NM_004985.5 *KRAS* (c.35G>A; p.Gly12Asp) VAF 23% from nevus sebaceous	Pathogenic: Mosaic RASopathy
10	RASopathy	NM_002834.5 *PTPN11* (c.1530G>C; p.Gln510His)	Pathogenic: Noonan syndrome
11	RASopathy	NM_006912.6 *RIT1* (c. 270G>T; p.Met90Ile)	Pathogenic: Noonan syndrome
12	RASopathy	NM_002834.5 *PTPN11* (c.923A>C; p.Asn308Thr)	Pathogenic: Noonan syndrome
13	RASopathy	NM_001354690.3 *RAF1* (c.1837C>G; p.Leu613Val)	Pathogenic: Noonan syndrome
14	Lymphedema distichiasis syndrome	NM_005251.3 *FOXC2* (c.443_449dup; p.Asp151Glyfs*314)	Likely pathogenic: lymphedema-distichiasis syndrome
15	RASopathy	NM_004333.4 *BRAF* (c.1802A>C; p.Lys601Thr)	Likely pathogenic: Cardiofaciocutaneous syndrome
16	RASopathy	NM_001654.4 *ARAF* (c. 640T>C; c. Ser214Pro) VAF 49% from pleura	Pathogenic: Mosaic RASopathy
69	Mosaic KRASopathy	NM_004985.5 *KRAS* (c.35G>A; p. Gly12Asp) VAF 17% from plexiform neurofibroma	Pathogenic: Mosaic RASopathy
70	RASopathy	NM_004333.4 *BRAF* (c.1799T>A; p.Val600Glu) VAF 8% from lymphatic malformation	Pathogenic: Mosaic RASopathy
18	Trisomy 21		Pathogenic: Down syndrome
19	Trisomy 21		Pathogenic: Down syndrome
20	Trisomy 21		Pathogenic: Down syndrome
21	Trisomy 21		Pathogenic: Down syndrome
22	Trisomy 21		Pathogenic: Down syndrome
23	Trisomy 21		Pathogenic: Down syndrome
24	22q11.2 del	arr[GRCh37]22q11.21(18,874,331–21,798,359)x1	Pathogenic: DiGeorge syndrome
25	22q11.2 del	arr[GRCh37]22q11.21(18919528–21460594)x1	Pathogenic: DiGeorge syndrome
27	PIEZO1	NM_001142864.3 *PIEZO1* (c.5190G>T; p.Trp1730Cys) paternally inherited; (c.5947G>C; p.Gly1983Arg) maternally inherited	VUS
28	PIEZO1	NM_001142864.3 *PIEZO1* homozygous (c.7289C>T; p.Pro2430Leu)	Pathogenic[a]
41	Metabolic disease	NM_000157.4 *GBA* Homozygous (c.1448T>C; p.Leu483Pro)	Pathogenic: Gaucher's Disease Type III
58	Metabolic disease	NM_000158.4 *GBE1* partial gene deletion; (c.1239delT; p.Asp413GlufsX23)	Pathogenic: Andersen disease

For mosaic cases, the variant allele frequency (VAF) and tissue type is included.
[a]Variant previously reported in Fotiou et al, 2015.

at presentation for lymphatic imaging was 3.7 years (range 14 days to 26 years). The most common clinical presentations of lymphatic disease were chylothorax (47%) followed by chylous ascites (24%). Consanguinity was not reported in the family history of any patients. Only one participant had a documented family history of lymphedema.

Genetic results

About a quarter of individuals (30 out of 115 or 26%) had a molecular etiology identified that could definitively account for their lymphatic presentation (Table 1). The most common category was RASopathies. There were 13 participants with germline RASopathies including 6 participants with pathogenic or likely pathogenic variants in *PTPN11*, 2 participants with *RAF1*, 2 participants with *RIT1*, 1 participant each with *SOS1*, *HRAS*, and *BRAF*. There were four individuals with mosaic RASopathies. One individual had a mosaic *BRAF* variant identified from previously excised abdominal lymphatic malformation. Another individual had a mosaic *ARAF* variant identified from pleura from a pleurodesis procedure. The variant was absent from blood on clinical exome sequencing. One individual had a mosaic *KRAS* variant identified from nevus sebaceous. Another individual had a mosaic *KRAS* variant identified from plexiform neurofibroma and verruca vulgaris but absent from blood. There was one individual with *FOXC2*-related Lymphedema Distichiasis syndrome. Chromosomal abnormalities were the next largest group. Six individuals had Trisomy 21. Two individuals had 22q11.2 deletion syndrome with *LZTR1* included in the deleted region as the most relevant candidate

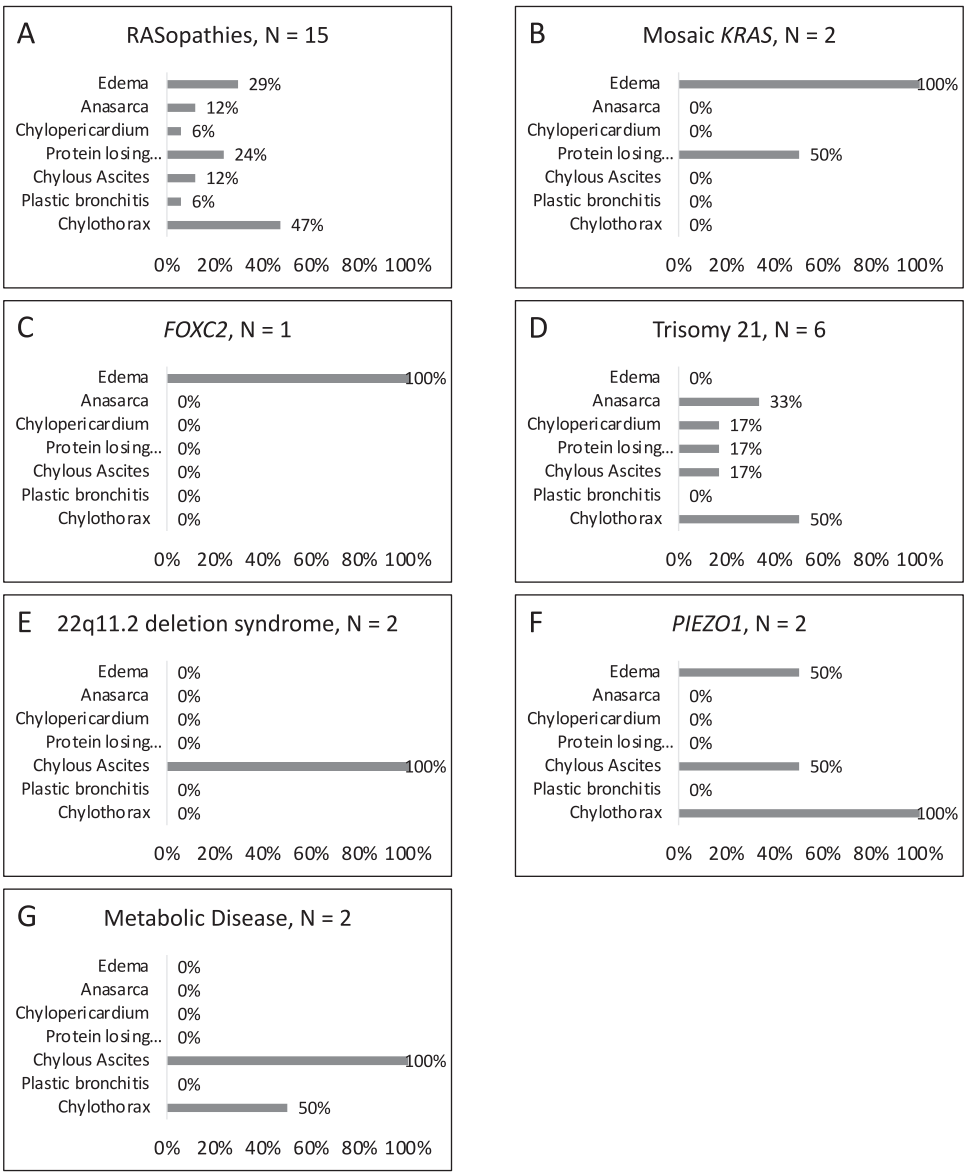

Fig. 2 The clinical phenotype of participants in the confirmed category. A RASopathies. **B** Mosaic *KRAS*-opathy. **C** *FOXC2*-related Lymphedema Distichiasis syndrome. **D** Trisomy 21. **E** 22q11.2 deletion syndrome. **F** *PIEZO1*-related Generalized Lymphatic Dysplasia. **G** Metabolic Disease including Andersen disease and Gaucher's Disease Type III. Protein losing protein losing enteropathy.

gene. Two individuals had biallelic variants in *PIEZO1*. There were two individuals with metabolic storage disorders – one each with Andersen Disease (Glycogen Storage Disease Type IV) and Gaucher Disease Type III.

Nine individuals were classified into the suspected category (Supplementary Table 2). Six individuals had VUSes in RAS/MAPK pathway genes, including two individuals with parentally inherited *LZTR1* variants. One individual had biallelic *ITGAD* variants of unknown significance, one individual had a *JAG1* likely pathogenic variant [11], and one individual had a 4q28.3q32.3 duplication [16].

Seventeen individuals were classified into the unknown etiology category (Supplementary Table 3). This was a highly heterogenous group which included individuals with confirmed cystic fibrosis, Dent's disease, Emanuel syndrome, G6PD deficiency, as well as several variants of unknown significance.

Genotype phenotype correlation

We evaluated the clinical features for the participants in the confirmed group (Fig. 2). Chylothorax was the major presenting feature for participants with germline or mosaic (*BRAF* or *ARAF*) RASopathy, Trisomy 21, or *PIEZO1* Generalized Lymphatic Dysplasia (47%, 50%, 100% of participants respectively). The participants with a mosaic *KRAS*opathy or Lymphedema Distichiasis syndrome had edema. Chlyous ascites was the main presenting feature for participants with 22q11.2 deletion syndrome or a metabolic disease.

Next, we evaluated the T2-W imaging and DCMRL for the participants (Fig. 3). We identified genotype phenotype correlations for some of the groups within the "confirmed etiology" category (Table 2). Similar to our previous findings, individuals with germline RASopathies as well as the individual with the *BRAF* mosaic RASopathy have large, dilated, beading lymphatics; retrograde posterior intercostal flow and pulmonary perfusion and abnormalities of the thoracic duct (Fig. 3A, C). Posterior intercostal flow was common in this group. In contrast, the individuals with somatic *KRAS* p.G12D variants had dilated channels and extensive abnormal perfusion in the lung but no posterior intercostal flow (Fig. 3B). The lymphatics in individuals with Trisomy 21 are characterized by disorganized

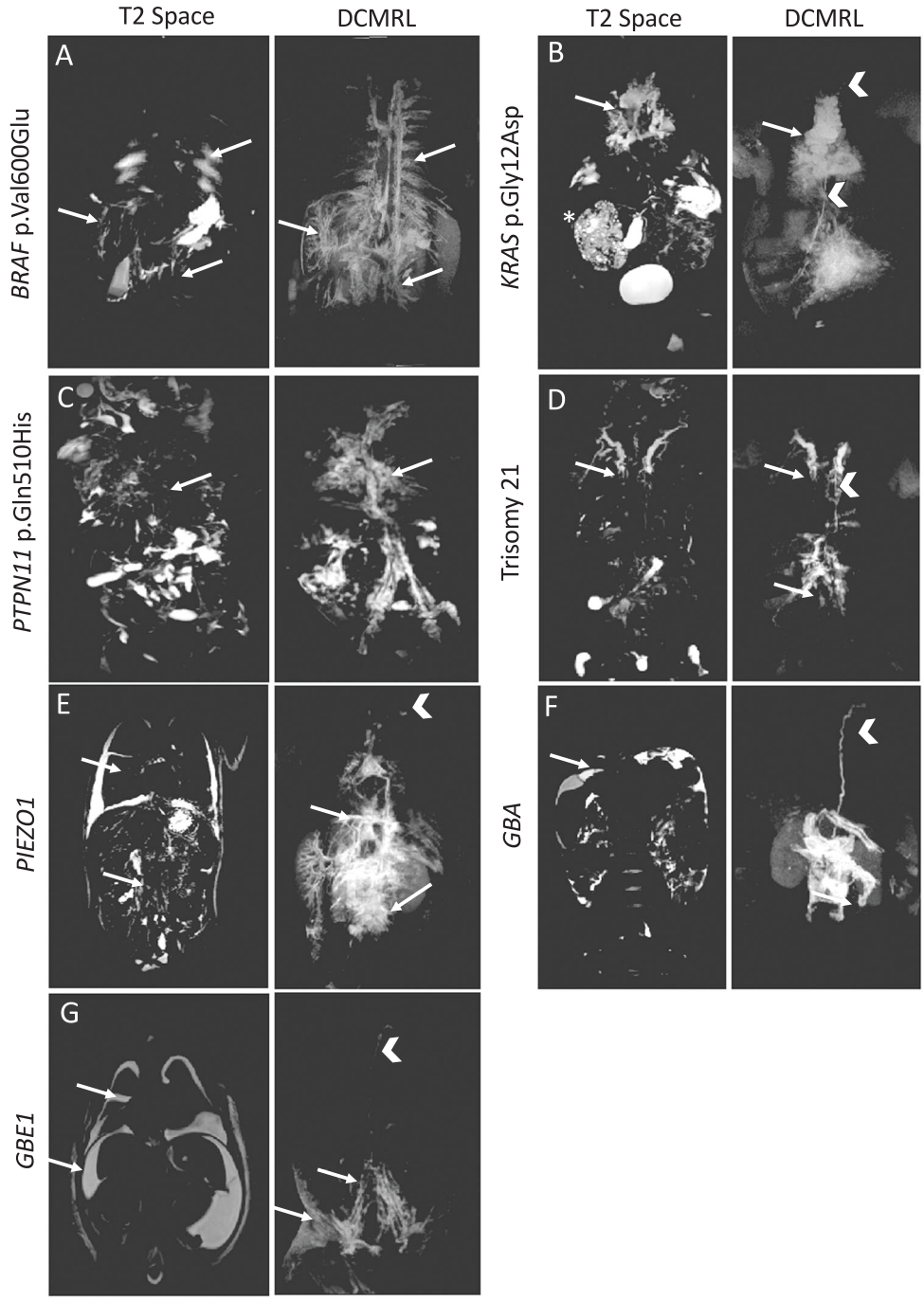

Fig. 3 Representative imaging findings based on genotype. T2 space and dynamic contrast MR lymphangiography from seven different genotypes illustrating lymphatic conduction abnormalities. **A** Mosaic *BRAF* (p.Val600Glu). T2 space shows significant edema in the intercostal, mesentery and liver lymphatics (left panel) (arrows) that correlates with abnormal perfusion patterns on intrahepatic DCMRL (right). Also note the abnormal lymphatic thoracic vessels with the absence of a normal thoracic duct. **B** Mosaic *KRAS* (p.Gly12Asp) There is edema on T2 space within the mediastinum and lungs (arrows). Patient also with cystic right kidney (asterisk). Intrahepatic DCMRL demonstrates correlation with mediastinal, pulmonary, and supraclavicular edema with perfusion of dilated lymphatic structures. Of note, this patient has a central thoracic duct (arrow heads), but it was not patent to the venous circulation on US contrast imaging. **C** Noonan syndrome (*PTPN11* p.Gln510His). T2 space imaging demonstrating mediastinal and intercostal edema predominately. With intranodal DCMRL these areas correlate with abnormal perfusion (arrows). Again, note there is no central thoracic duct, but persistent pulmonary and intercostal perfusion. **D** Trisomy 21. T2 space imaging with edema in the supraclavicular (and superior mediastinal lymphatics (arrows)). On intrahepatic DCMRL there is retrograde flow into retroperitoneal lymphatics, intercostal, mediastinal, pulmonary, and supraclavicular perfusion (arrows). There is a patent thoracic duct that courses to the left venous angle (arrowhead). **E** *PIEZO1*. T2 space shows bilateral pleural effusions, pulmonary, and retroperitoneal edema (arrows). Intrahepatic DCMRL shows extensive flow to the hepatic capsular lymphatics with extension into the mediastinum and pulmonary lymphatics (arrows). There is also retrograde flow into the retroperitoneal lumbar and mesenteric lymphatics. There is a small thoracic duct seen coursing to the left venous angle (arrow head), patent on follow up imaging. **F** Gaucher's Disease Type III. T2 space notable for ascites. Intrahepatic DCMRL shows retrograde perfusion to retroperitoneal lumbar lymphatics and mesentery (arrows). The thoracic duct is mildly dilated and tortuous as it courses to the left venous angle (arrowhead). **G** Andersen disease. T2 space imaging with significant ascites, pleural effusions, and anasarca (arrows). With intranodal DCMRL, there is extensive dermal perfusion and dilated retroperitoneal lymphatics. A thoracic duct is present and mildly dilated and tortuous (arrowhead).

Table 2. Characteristic lymphatic imaging findings by genotype.

Genotypic category (*N*)	DCMRL findings
Germline Rasopathies (13), *BRAF* Mosaic Rasopathy (1), *ARAF* Mosaic Rasopathy (1)	Dilated, tortuous, beading lymphatics Posterior intercostal flow Malformed or absent central thoracic duct
Somatic *KRAS* p.G12D variants (2)	Dilated channels and extensive abnormal perfusion in the lung
Trisomy 21 (6)	Disorganized central lymphatics Dilated channels in the head and neck Some posterior intercostal flow but less than RASopathies
PIEZO1 lymphatic dysplasia (2)	Perfusion of the hepatic capsule with connections to the peribronchial lymphatics Disorganized retrograde flow in the abdomen Dermal backflow

central lymphatics and dilated channels in the head and neck (Fig. 3D). Some individuals had some posterior intercostal flow, but generally there were fewer intercostal channels affected compared to the main RASopathies group. Individuals with *PIEZO1* lymphatic dysplasia have lymphatics that are characterized by perfusion of the hepatic capsule with bilateral connections to the peribronchial lymphatics (Fig. 3E). Disorganized retrograde flow in the abdomen and dermal backflow are also seen. There were not characteristic imaging findings for the two individuals with 22q11.2 deletion syndrome. The participant with *FOXC2* Lymphedema- Distichiasis syndrome did not have a thoracic duct identified by multicompartment DCMRL (mesenteric, hepatic, and groin lymph node injections). There appeared to be an absence of lymphatic channels above the level of the inguinal ligament leading to conventional lymphangiography. Together, the findings suggested multiple lymphovenous connections.

We also quantified the abnormalities on T2-W imaging and DCMRL (Supplementary Figs. 1–3). Within the RASopathies category, the most common T2-W imaging findings were pleural effusion (73%) and ascites (53%). On DCMRL, more than half of individuals (67%) exhibited perfusion to the lung. Other common areas of perfusion were the mesentery (33%), the mediastinum (27%), intestine (27%) and dermal backflow (27%). Only one individual in this group had a normal appearing thoracic duct. The most common thoracic duct finding on DCMRL was the absence of a central thoracic duct (33%), though two participants had previous ligation or embolization of their thoracic duct. In the mosaic *KRAS* group, both individuals had perfusion to the retroperitoneum on DCMRL. Within the Trisomy 21 category, the most common T2-W imaging findings was ascites (67%). On DCMRL, the most common areas of perfusion were the lung (67%), the mediastinum (67%), and mesentery (67%). Half of the participants had a normal appearing thoracic duct and one third of participants had bilateral thoracic ducts. Within the 22q11.2 deletion syndrome category, one individual received MRI imaging and the other received direct lymphangiography. T2-W imaging demonstrated ascites. DCMRL or direct lymphangiography demonstrated lymphatic perfusion of the peritoneum and the retroperitoneum. Within the *PIEZO1* category, both individuals had mediastinal edema on T2-W imaging. Both individuals with metabolic disease had ascites. Imaging findings for the individuals in the suspected and unknown categories are presented in Supplementary Tables 2 and 3.

DISCUSSION

In this study, we established preliminary genotype-phenotype correlations for individuals with CCLA. First, we defined the spectrum of molecular diagnoses seen in individuals with CCLA and established the yield of clinical genetic testing for this cohort. We also expanded the lymphatic phenotypes associated with specific genotypes and quantified the central lymphatic findings as seen on T2-W imaging and DCMRL.

Our results show that about a quarter of individuals with CCLA have an underlying molecular diagnosis. A benefit of our clinical cohort is that it allows us to present all known causes that may be associated with CCLA. However, we also found that only about a quarter of those who received care through our institution within the study period had a clinical genetics evaluation. It is also possible that individuals with more complex presentations or syndromic appearance are more likely to receive a clinical genetics evaluation and thus it could be that our molecular diagnosis rate is an overestimate due to selection bias. Additionally, because this was a retrospective study, our data was reliant on the clinical genetics evaluation. For example, our molecular diagnosis yield could potentially be increased by sampling of tissue for somatic mosaicism or exome sequencing as follow up for all individuals in a research setting with subsequent clinical validation. Regardless, our data indicate that clinical genetics is an essential component of the care for individuals with CCLA and currently, genetic testing is recommended for all patients with CCLA or other forms of lymphatic disease.

Our data show that the most common syndromes that have CCLA as a feature including RASopathies (both germline and mosaic), chromosomal abnormalities, *PIEZO1* lymphatic dysplasia, and metabolic disorders. Consistent with previous results, RASopathies was the largest category [7]. Unlike previous studies of Noonan syndrome noting a preponderance of males with lymphatic issues, our RASopathy group showed a similar sex distribution with nine females and eight males [14]. Although this confirms the well-established role of RAS/MAPK in abnormal vascular and lymphatic vessel growth, these results could also reflect greater utilization of Noonan's panel testing which was the most common disease panel ordered in our cohort. Importantly, our results also provide additional candidate regions of the genome as well as candidate genes that may be associated with CCLA. Further research is needed evaluate the causal role of these genomic regions that may provide novel insights into lymphatic development and disease. In our study, we excluded participants as "acquired" if the participant presented with lymphatic symptoms after a surgical intervention. This does not account for the possibility that there may be lymphatic dysfunction or malformations present but not clinically apparent before surgery. Further work is needed to understand the true natural history and genetic contribution to CCLA in participants with complex congenital heart disease requiring repair.

This study builds on our previous work evaluating the genotype-lymphatic phenotype correlation in Noonan syndrome [7]. However, there are some limitations. Due to the low number of individuals within each of the confirmed etiology category, only limited conclusions can be drawn regarding lymphatic conduction patterns. This is partially due to lack of clinical genetic evaluation in almost three quarters of the individuals. Additionally, some individuals in this study had their lymphangiogram after previous intervention including thoracic duct ligation or embolization, which likely changes the flow pattern. Finally, not all individuals in this study had intrahepatic DCMRL, which our group has now found is an important diagnostic

evaluation for CCLA [18]. Standardization of evaluation will be essential in future studies, as previously noted [8]. Despite these limitations, we confirmed our previous genotype-phenotype association in RASopathies and discovered novel characteristic features for mosaic *KRAS*-opathies, Trisomy 21, and *PIEZO1*-related lymphatic dysplasia. In the absence of typical characteristic features and detailed patient history, lymphatic system mapping can guide molecular analysis for individuals with CCLA.

In summary, our work further defines subcategories of CCLA as a first step towards "precision lymphology." We suggest nomenclature that involves both the genotype (if known) and phenotype for precise diagnosis (e.g mosaic *KRAS* related-CCLA) [19]. This should be considered for updated ISSVA classifications of CCLA. This work is essential for more precisely defining the natural history of this heterogenous group of lymphatic disorders. Further studies are necessary to strengthen our genotype-phenotype correlations.

DATA AVAILABILITY

The data that support this study are available in the tables and Supplementary Tables. Additional data is not available due to privacy restrictions.

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AUTHOR CONTRIBUTIONS

Conceptualization: YD, SES; Data curation: ML, CS, YD, SES; Formal Analysis: ML, CS, DL, SES; Funding acquisition: YD, SES; Investigation: ML, CS, DMB, DL, NR, EP, CS, EHZ, YD, SES; Methodology: ML, SS; Project administration: ML, YD; Supervision: YD, SES; Validation: ML, CS; Visualization: ML, CS, YD, SES; Writing (original draft): ML, SES; Writing (review & editing): ML, CS, DL, HH, YD, SES.

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COMPETING INTERESTS

HH and The Children's Hospital of Philadelphia are equity holders in Nobias Therapeutics Inc., developing MEK inhibitor therapy for complex lymphatic anomalies. Other authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Children's Hospital of Philadelphia Institutional Review Board approved a registry and biorepository study (PI YD). Informed consent was obtained from all participants as required by the IRB.

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