Analysis of gene PIK3CA mutation in patients with lymphatic malformations

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Background

Lymphatic malformations (LM) are benign lesions, composed of dilated, fluid filled lymphatic cysts, which result from developmental abnormalities in the lymphatic system. While often inconspicuous at birth, they tend to progress during childhood, presenting with symptomatic manifestations prompting medical attention. LMs can occur as isolated or diffuse, sometimes as part of syndromes (e.g. Klippel-Trenaunay syndrome, CLOVES syndrome). Lesions may cause deformations of the body, impairment of physical development of the patient, disorders in physiological functions, or can even be life-threatening. Lymphatic malformations commonly induce pain and functional limitations, potentially impacting daily activities and social functioning, particularly in children facing stigmatization.

Current therapeutic options remain limited, comprising of sclerotherapy, compression techniques, surgical excision, laser therapy, and in some cases targeted therapies, which are relatively new. However, these approaches demonstrate variable efficacy, often insufficient for diffuse lymphatic malformations in pediatric patients.

Recently genetic studies have shown that LM are probably caused by somatic mutation in phosphatidylinositol-4,5-bisphosphate-3-kinase catalytic subunit alpha (PIK3CA) gene. The correlation between the occurrence of the malformations and the PIK3CA somatic mutations seems promising in search for targeted therapies.

Purpose

Analysis of the occurrence and type of gene PIK3CA mutation in patients with LM.

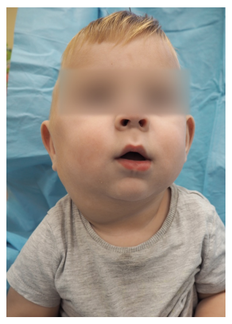
Materials and Methods

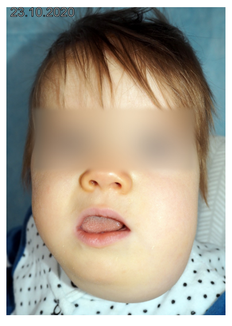
- 36 patients with LM included in the research (29 retrospectively, 7 prospectively)
- Inclusion criteria
  - Age <18 y.o. at the moment of diagnosis
  - Patients with isolated or diffuse lymphatic malformations
  - Patient with CLOVES syndrome
  - Patients with Klippel-Trenaunay syndrome
- Tissue samples were obtained during clinically indicated procedures; they were formalin-fixed paraffin embedded (FFPE) and stored as paraffin blocks. Part of the paraffin blocks were cut into sections using microtome. We used 2-4 sections per patient, each 5-10 um thick.
- Purification of genomic DNA from FFPE was made using QIAamp DNA FFPE Tissue Kit, using ddPCR method search for the PIK3CA gene mutation in hotspots p.E542K (c.1624G>A), p.E545K (c.1633G>A), p.E545G (c.1634A>G), p.H1047R (c.3140A>G), p.H1047L (c.3140 A>T) was made (CORELAB, Medical University of Lodz)
- Statistical analysis
  - Samples with three or more positive mutant droplets were considered positive, as recommended by the best practice guidelines for rare mutation detection
  - The Shapiro-Wilk test was performed to test for normal distribution. Continuous variable are presented as median with the values of the lower and upper quartiles (25-75 percentile). Categorical variables are presented as numbers with an appropriate percentage

Results

Patient Characteristics	n(%)
Total patients	36
Male	12 (33,33%)
Female	24(66,67%)
Age at the time of surgery (years)	
Median	3
Range	0,5-17
Type of malformation	
Isolated LM	19 (52,78%)
Diffuse LM	17 (47,22%)
Location	
Head-neck	12 (33,33%)
Trunk	18 (50%)
Limb	4 (11,11%)
Trunk, limb	1 (2,78%)
Head-neck, trunk, limb	1 (2,78%)
Mutation	**# of patients with mutation**
PIK3CApH1047R	4 (11,11%)
PIK3CApE545K	4 (11,11%)
PIK3CApE542K	7 (19,44%)
PIK3CApE545G	0 (0%)
PIK3CApH1047L	4 (11,11%)

Figure 1. Patient characteristics





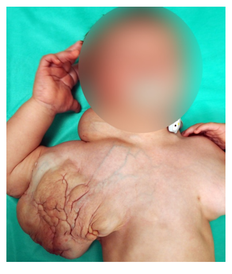


Figure 2. Patients with lymphatic malformations

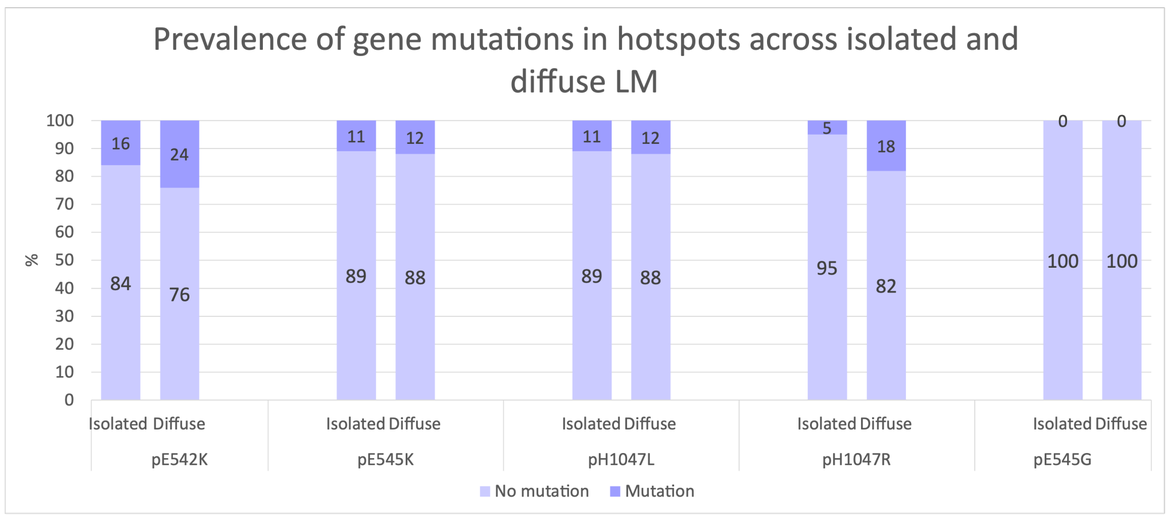


Figure 3. Prevalence of gene mutations in hotspots across isolated and diffuse LM

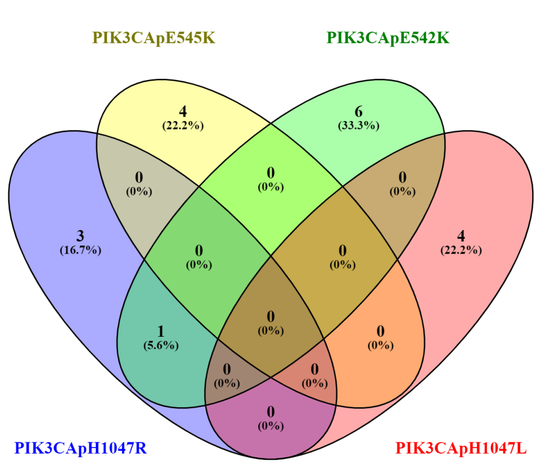


Figure 4. Venn diagram of mutations among patients

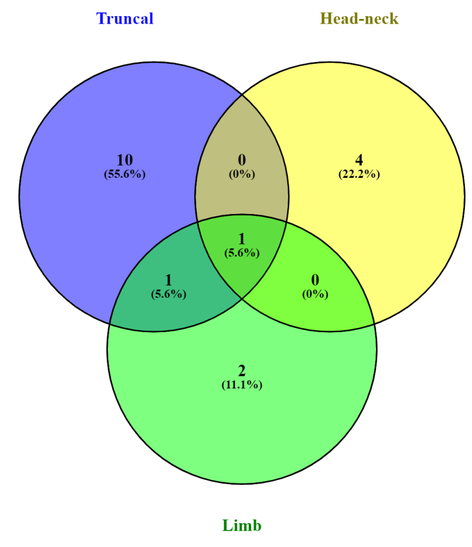


Figure 5. Location of the malformation in the patients with mutation

Conclusions

Conclusions will be presented upon completion of statistical analyses.