Absence of phenotypic predictors of *PIK3CA* mosaicism in a cohort of children with vascular malformations with and without overgrowth

Polubothu M, Knox R, Al-Olabi R, Eastwood D, Eleftheriou G, Gholam K, Glover M, Kangesu L, Mahon C, Martinez A, Shaw L, Sivakumar B, Smith G, Syed S, Tasani M, Parker V, Semple R, Kinsler VA

Background
PIK3CA Related Overgrowth Spectrum (PROS) is an umbrella term describing the known and emerging clinical entities associated with post-zygotic *PIK3CA* mutations[1]. More recently *PIK3CA* mutations have been described in venous malformations without overgrowth (Vikkula M group paper AJHG end 2015). Many individuals present with overlapping features of syndromes previously thought to be clinically distinct. Accurate phenotypic and genotypic description of the clinical spectrum of *PIK3CA* mutation mosaicism is necessary to improve our understanding of the condition, and to attempt to identify individuals likely to benefit from appropriate genetic testing.

Objectives
To look for phenotype-genotype associations in a cohort of patients with vascular malformations in a tertiary paediatric dermatology department.

Methods
Since January 2015 targeted next generation sequencing of a panel of known overgrowth genes has been offered systematically to all patients who have attended our department with a diagnosis of vascular malformation and/or of overgrowth, where affected tissue has previously been saved, or where the patient consents to a new punch skin biopsy. Thus far 50 patients have had phenotyping and sequencing, for whom results are available currently on 33 (14 male). DNA was extracted from samples using standard methods. Sequencing has a sensitivity of 1% for fresh and 5% for paraffin-embedded samples. Multiple logistic regression was used to model *PIK3CA* mutation status on the basis of phenotypic variables.

Results
Median age at presentation was 0.9 years, median follow up 8.6 years. During the period of follow up 42% of patients had overgrowth of body and/or limb, 7% had macrocephaly, 57% had a visible superficial component to the vascular malformation in the form of capillary malformation or lymphatic malformation, 11% had epidermal naevi. Of the 13 patients who had clotting studies/D-dimers 69% of these were abnormal. 69% of the 13 children who had clotting studies had abnormal coagulation times or elevated D dimers. *PIK3CA* mutations were detected in affected tissue of 13/33 patients (39%), and absent from blood in all 10 patients where this was also sequenced. The mutant allele frequency in tissue biopsies was between 1.2% and 26%. *AKT1* mosaicism was found in one patient with a mild phenotype not sufficient for a diagnosis of Proteus syndrome. Thus far

no clinical phenotypic variables have been statistically predictive of *PIK3CA* mosaicism.

Conclusions

39% of patients in this cohort of children with vascular malformations with and without overgrowth carry *PIK3CA* mutations in affected tissue. Our findings confirm the broad phenotypic spectrum reported with mutations in *PIK3CA*. Thus far we have not identified any phenotypic predictors of mutational status, however larger numbers and more detailed phenotyping may alter this result. Sensitive genotyping is likely to be key in the choice of novel targeted therapeutic strategies for these children.

References:

1) Kleppler-Noreuil et al. Am J Med Genetics. 2015.