Supplementary Information

Mosaic Overgrowth with Fibroadipose Hyperplasia is Caused by Somatic Activating Mutations in PIK3CA

Marjorie J Lindhurst*, Victoria ER Parker*, Felicity Payne, Julie C Sapp, Simon Rudge, Julie Harris, Alison M Witkowski, Qifeng Zhang, Matthijs P Groeneveld, Carol E Scott, Allan Daly, Susan M Huson, Laura L Tosi, Michael L Cunningham, Thomas N Darling, Joseph Geer, Zoran Gucev, V. Reid Sutton, Christos Tziotzios, Adrian K Dixon, Timothy Helliwell, Stephen O'Rahilly, David B Savage, Michael JO Wakelam, Inês Barroso#, Leslie G Biesecker#, Robert K Semple#

co-corresponding

*These authors contributed equally to this work.

Marjorie J Lindhurst & Victoria ER Parker

Correspondence:

Dr. Leslie G. Biesecker, NIH – National Human Genome Research Institute, 49 Convent Drive Room 4A56, Bethesda, MD 20892

T: 301-402-2041

F: 301-402-2170

E: leslieb@helix.nih.gov

OR

Dr. Robert K. Semple, University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Box 289, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK.

T: +44 1223 769 035

F: +44 1223 330 598

E: rks16@cam.ac.uk

OR

Dr. Inês Barroso, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SA, UK.

T: +44 (0)1223 496 928

F: +44 (0)1223 494 919

E: ib1@sanger.ac.uk

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Description of recruitment and phenotyping.

Supplementary Table 1: Summary of *PIK3CA* Mutation Burdens Tissues and Cells Tested

Patient	Anatomical Source[1]	Tissue Mutation level (%)[2]	Cultured cells Mutation level (%)[3]
C1	Adipose tissue – left leg	39	-
Leu[4]	Muscle[5] – left leg	33	-
	Fibrous tissue – left leg	32	-
	Skin – left leg	24	50
	Bone – left leg	8	-
	Skin – left arm	-	0
	Blood	0	-
C2	Right arm	49	-
Leu	Right thumb	18	-
N7	Articular cartilage - left foot	-	32-34
Arg	Adipose tissue - left foot	-	33
	Bone - left foot	-	31-35
	Skin - left foot	-	30-34
	Muscle - left foot	-	12-19
	Deep tissue - left foot	-	2-7
	Blood	0	-
N45	Skin - ankle	-	<1-2
Arg	Blood	0	-
N68	Skin - left second finger ulnar surface	-	<1-5
Arg	Blood	0	-
N99	Growth plate - left second toe middle phalanx	22	-
Arg	Growth plate - left third toe proximal phalanx	27	-
	Growth plate - left third toe middle phalanx	9	-
	Growth plate - left second toe proximal phalanx	31	-
	Growth plate - left second toe distal phalanx	25	-
	Growth plate - left third toe middle phalanx	14	-
	Skin - webbing between second and third toes	-	29-32
	Blood	0	-
N104	Skin - left hand	-	1-3
Arg	Blood	0	
N108	Skin – left leg	5	
Leu	Adipose tissue – left leg	4	
	Blood	0	
N109	Skin – dorsal left foot	12	30-31
Arg	Blood	0	-
N110	Skin - medial tip of second left toe	7	27
Arg	Blood	0	

[1]Description of the source tissue for mutation analyses.

[2]Percentage *PIK3CA* c.3140A>T (p.His1047Leu) as determined by BsaBI or MseI restriction assays or c.3140A>G (p.His1047Arg) as determined by BmgBI restriction assay in DNA extracted directly from tissue.

[3]Percentage *PIK3CA* c.3140A>T or c.3140A>G as determined by BsaBI or BmgBI assays respectively, in DNA extracted from cells cultured from tissue specimens. Range indicates mutation levels if multiple DNA extractions were performed.

[4]Leu indicates p.His1047Leu mutation and Arg indicates p.His1047Arg mutation.

[5]Macroscopic appearance of muscle but heavy infiltration with adipocytes microscopically**.**

Supplementary Table 2: Primers and enzymes used for custom restriction assay

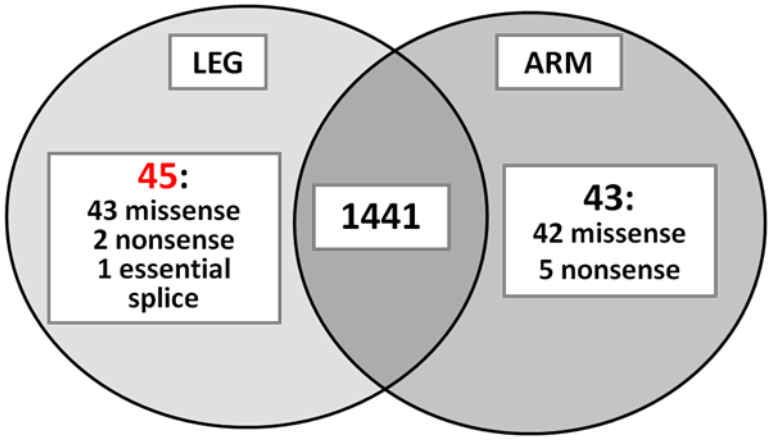
Codon 1047 status	Forward Primer	Reverse Primer	Enzyme
Wild type	(6-FAM)-GGTCTTTGCCTGCTGAGAGT	TGAGCAAGAGGCTTTGGAGT	BsaBI (Fermentas)
p.His1047Arg	(6-FAM)-CTGAGCAAGAGGCTTTGGAG	AGTGTGGAATCCAGAGTGAG	BmgBI (New England Biolabs)
p.His1047Leu	(6-FAM)-TGATGCTTGGCTCTGGAATG	TTTGTTGTCCAGCCACCATTA	MseI (New England Biolabs)

Supplementary Table 3: Primary antibodies used for immunoblotting

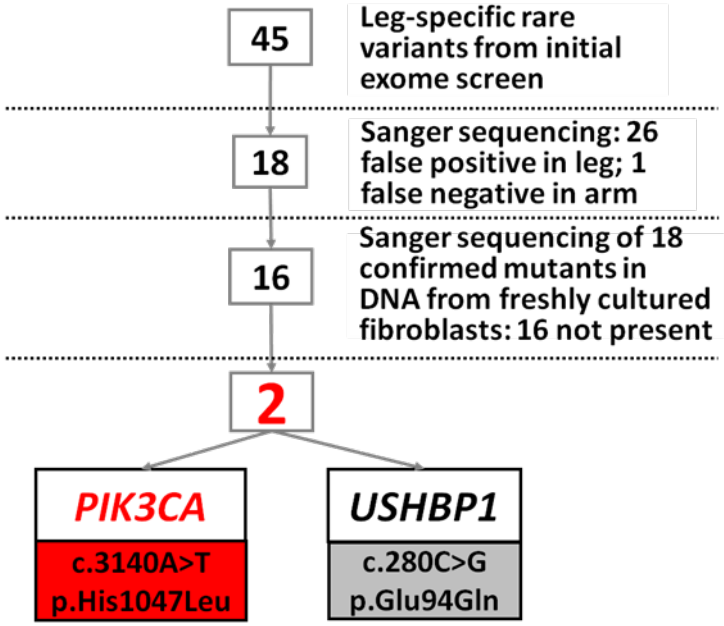
Primary antibody	Manufacturer/ cat#
pAKT (Ser474)	Cell Signaling Technology/4060
pAKT (Thr309)	Cell Signaling Technology / 4056
Total AKT	Cell Signaling Technology / 2920
pGSK3	Millipore/ 05-413
p70 S6 kinase (Thr389)	Cell Signaling Technology / 9234
pERK1/2	Cell Signaling Technology / 4370
Calnexin	Cell Signaling Technology /2679

Supplementary Figure 1: Summary of Exome Sequencing and Genetic Follow Up Studies in Patient C1. A. Forty-five novel variants were called as being exclusive to leg cells by whole exome sequencing. **B.** Further Sanger sequencing of all variants called as being leg-specific identified 26 false positive calls (i.e. the mutation was not found in leg DNA) and 1 false negative call in the arm (i.e. the mutation was found in both leg and arm DNA). DNA was then extracted from early passage fibroblasts obtained from a fresh cutaneous biopsy and this ruled out a further 16 mutations, leaving 2 candidate pathogenic mutations.

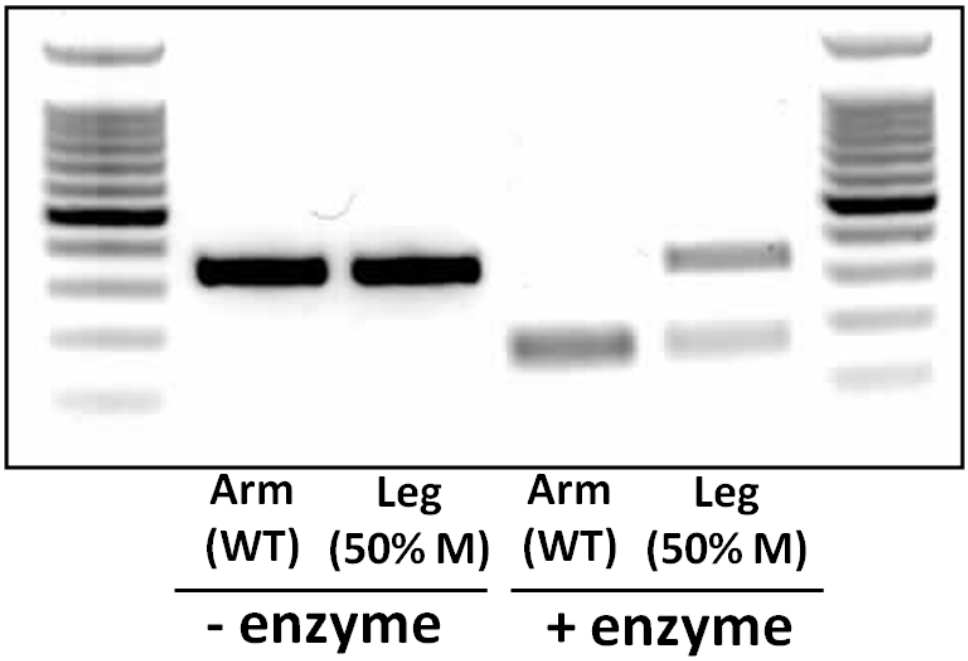
A



B



Supplementary Figure 2: BsaBI digestion of mutation spanning *PIK3CA* amplicon in arm and leg cells from patient C1. WT: wild type, M: mutant. See Supplementary Table 2 for primers and enzymes used in this assay.



Supplementary Note: Clinical Data

The subjects were recruited through referring clinicians for their unusual pattern of overgrowth to join ongoing research studies of LGB & RKS. These research studies were reviewed and approved by the appropriate IRB/Ethics committee and the participants provided informed consent or assent, as stipulated by the review committees. The clinical evaluations of the patient varied according to their manifestations. These evaluations included physical examination and imaging studies including plain radiography, magnetic resonance imaging, and other techniques. Samples were collected from affected and unaffected areas of the participants, most commonly by punch biopsy.