90% *TSC1/TSC2* mutation detection rate in TSC patients without mutation identified in commercial labs

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Introduction: Our past Massively Parallel Sequencing (MPS) studies have shown that mosaicism is a common phenomenon in TSC patients who had 'no mutation identified' (NMI) by conventional testing.

Materials and Methods: We used MPS for analysis of 144 samples [different TSC tumors/tissues/fluids: facial angiofibroma (FAF), ungual fibroma, shagreen patch, hypomelanotic macule, angiomyolipoma (AML), normal skin, buccal swab, saliva, blood, semen and amniotic fluid] from 30 NMI TSC patients (median age:33), including 5 with mild TSC manifestation (2 clinical features only).

Mutations were detected using hybrid-capture MPS and validated by our new MPS Multiplex High-sensitivity PCR Assay (MHPA) method.

Hybrid capture MPS of entire *TSC1/TSC2*
- median coverage: 740x;
- sensitivity: 0.5% variant allele frequency (VAF)

samples: 1-2 FAFs or AML lesions per patient

validation of the identified mutations

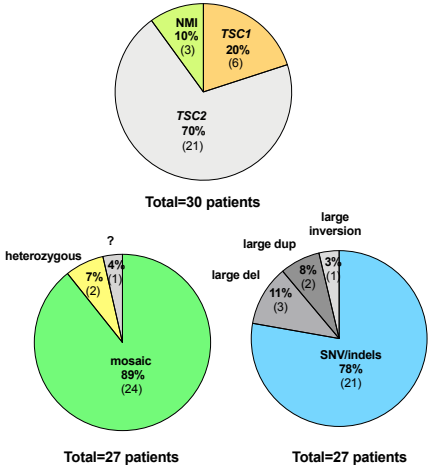
Targeted amplicon MPS

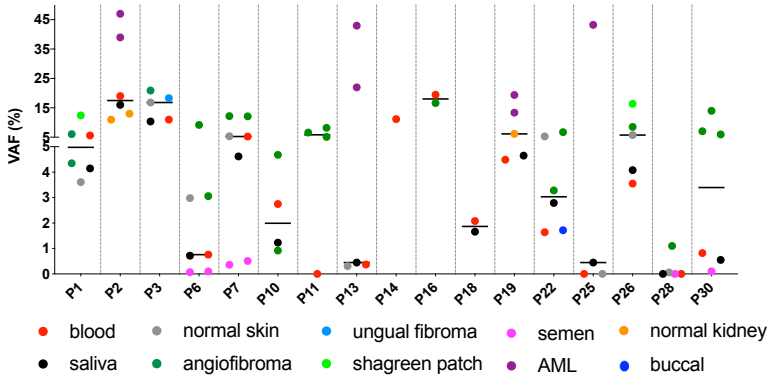
Multiplex High-sensitivity PCR Assay (MHPA) with Unique Molecular Identifier (UMI) barcoding for error suppression
- median coverage: 20,000x;
- sensitivity: 0.05% variant allele frequency (VAF)

Allele-specific PCR/Sanger sequencing

samples: all available per patient

Results: *TSC1/TSC2* mutations were identified in 27 of 30 patients (90%) [21(78%) in *TSC2*; 6(22%) in *TSC1*]; 24 patients had mosaicism [blood VAF:0-19%, median:2.8%]. VAFs of the identified mosaic mutations were enriched in TSC tumors in comparison with normal tissues and fluids.



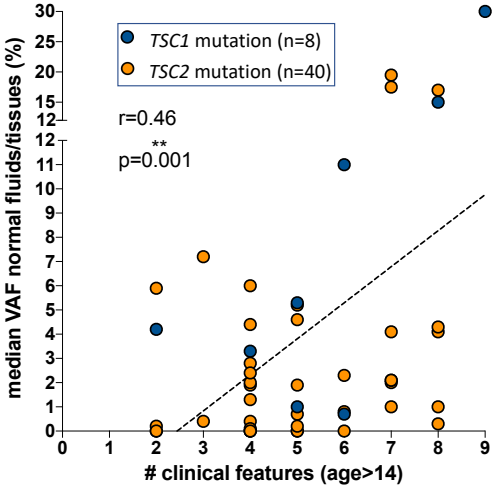


We identified novel *TSC1/TSC2* mutations and an individual with minimal TSC features and two unique *TSC2* mutations in each of AML and FAF, suggesting that both tumor events were sporadic.

Number of TSC-related clinical features was highly variable and correlated with mosaic *TSC1/TSC2* VAF (*Klonowska et al., Giannikou K et al., Genet Med, 2019, Tyburczy M et al., PloS Genet, 2015*).

Patient ID	Mutation type	Mutation name	Gene	Mosaic / heterozygous	Tissues / fluids with mutation	VAF range
P23	*de novo* deep intronic indel	c.363+749delG	*TSC1*	heterozygous	white spot blood saliva	50%
P21	inversion (221 kb)	inv_*TSC2* _exon31 – *ABCA3* _exon13	*TSC2* – *ABCA3*	either mosaic or heterozygous	amniotic fluid	-
P2	duplication (39 bp)	c.226-23_241dup (p.Leu81Argfs*7)	*TSC2*	mosaic	AML1 AML2 normal kidney blood saliva	12-47%
P24	duplication (19 kb)	dup_*TSC2*_ ex3-*TSC2* _ex15	*TSC2*	mosaic	AML1 AML2 blood saliva ungual fibroma FAF1 FAF2	-
P12	large deletion (166 bp)	c.4990-93_5063del (*TSC2* _ex38)	*TSC2*	mosaic	L nasal FAF R nasal FAF nasolabial fold FAF saliva normal skin blood	4-5%
P27	large deletion (11 kb)	del_*TSC2* _ex26 – *TSC2* _ex38	*TSC2*	mosaic	blood buccal swab	14%
P17	dinucleotide substitution	c.1248_1249CC>TT (p.Gln417*)	*TSC2*	mosaic	FAF	2%
	single nucleotide substitution	c.3319G>T (p.Glu1107*)	*TSC2*	mosaic		2%
	indel	c.4345_4346insC (p.Arg1451Profs*73)	*TSC2*	mosaic	AML	16%
	single nucleotide substitution	c.4712A>G (p.Tyr1571Cys)	*TSC2*	mosaic		21%

Mosaicism:
situation in which different cells in the body have a different genetic make-up; occurs due to a postzygotic mutation event resulting in a clonal population of cells in the body with the variant, mixed in with a larger fraction of cells that do not have the variant



Conclusions: We have developed an analysis approach for very sensitive *TSC1/TSC2* mutation detection that is capable of a 90% detection rate on NMI cases and define the spectrum of mosaic mutations and associated clinical features in greater detail than previously.

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