

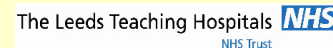
Screening of MKS1, TMEM67 (MKS3) and selected founder mutations detects biallelic mutations in a significant proportion of patients with Meckel Syndrome



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Introduction

Meckel syndrome (aka Meckel-Gruber syndrome, MKS; MIM 249000) is a rare, lethal autosomal recessive disorder characterised by developmental anomalies of the brain and nervous system, postaxial polydactyly, hepatic developmental defects, encephalocele and cystic dysplasia of the kidneys. Other associated features include microphthalmia, cleft lip and palate, heart defects, genital abnormalities, bowing of long bones, and complete or partial *situs inversus*.

MKS is part of the ciliopathy spectrum of diseases and has some genetic and clinical overlap with Joubert syndrome, nephronophthisis and Bardet-Biedl syndrome.

To date, 7 genes have been implicated; MKS1, TMEM216, TMEM67, CEP290, RPGRIP1L, CC2D2A and NPHP3. In addition to the true, Mendelian causative alleles reported in these genes, there is significant evidence for modifying alleles in MKS and other cilia-related genes.

Mutation detection strategy

Our initial service provided accredited sequence confirmation for mutations detected in research projects, with provision for familial carrier testing and prenatal analysis. Assays can be rapidly set up "bespoke" for individual families in any of the known MKS genes, and utilise universal M13-derived sequencing primers.

Since 2009, full gene screening for MKS1 and TMEM67 (MKS3) has been in operation; these two genes include a large proportion of mutations so far reported in the literature (although frequency data is difficult to interpret due to the rarity of the syndrome), with common founder mutations in the Northern European (MKS1) and Pakistani (TMEM67) populations detected in several diagnostic screens. In 2011, information collated from the local research group and published mutation data has allowed for some targeted testing for additional specific mutations in several other MKS genes that have been implicated as possible founder effects in both the Northern European population and the local Mirpuri Pakistani population (see table 1).

Referrals can be set up for any or all of the services offered (MKS1, TMEM67, population-specific "common" mutations). Literature suggests polydactyly and encephalocele are less common in MKS3 than MKS1, so TMEM67 screening may be preferable in probands lacking these features.

Conversely, the relative frequency of the MKS1-Fin_{major} mutation (c.1408-34_1408-6del) in cases of European origin suggests MKS1 as a start-point for Caucasian patients with a more classical clinical picture. These decisions are clinically driven and made in conjunction with users and the local Clinical Genetics Department.

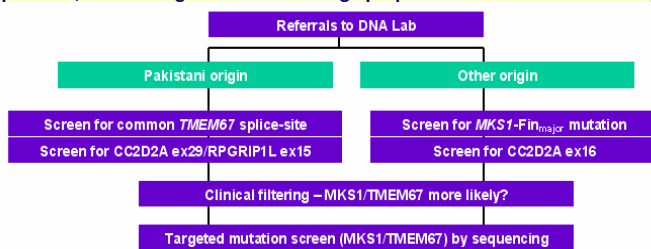


Fig.1: Test strategy

Screening results

To date, 13 families (either probands or parents of affected fetuses) have been screened in full for MKS1 mutations, 9 for TMEM67 mutations (see table 2). Additional patients have received targeted screening in either gene for common founder mutations (in one case, a common TMEM67 founder mutation was detected in one TOP sample and reported within 10 days of receipt), and retrospective testing for some other founder mutations has been performed in requested cases. 4 patients have been found to have founder mutations (homozygous in all but one compound heterozygote case; see table 1). An additional patient (plus sibling) with a milder phenotype was found to be compound heterozygote for two previously undescribed TMEM67 mutations (nonsense + missense; see fig.2).

Gene:	Mutation:	Consequence:	N (research confirmations):	N (diagnostic screens):	Founder population:	Reference:
MKS1 (FLJ20345)	Fin _{major} *	Splice defect	1	2 (1 het, 1 homoz.)	Finnish/N. European	(Carrier freq. Germans 1 in 240, Finns may be higher) Kyttälä et al 2006, Nat Genet, 38(2), 135-6.
TMEM67 (MKS3)	c.870-2A>G	Splice defect	1	0	Pakistani (?Mirpuri)	Local research data (several families; two founder muts may account for up to 40% of incidences in this population).
TMEM67 (MKS3)	c.1575+1G>A	Splice defect	3	1	Pakistani (?Mirpuri)	Local research data (several families; two founder muts may account for up to 40% of incidences in this population).
CC2D2A (MKS6)	c.1762C>T	Nonsense	0	1	Finnish/N. European	(575 Finnish normal controls, carrier freq. 0.5%) Tallila et al 2008, Am J Hum Genet, 82(6), 1361-7.
CC2D2A (MKS6)	c.3540delA	Frameshift	1	0	Pakistani (?Mirpuri)	Local research data (2 families).
RPGRIP1L (MKS5)	c.1945C>T	Nonsense	1	0	Pakistani (?Mirpuri)	Local research data (2 families).
NPHP3 (MKS7)	c.2694-2_2694-1del	Splice defect	0	0	?European	Bergmann et al 2008, Am J Hum Genet, 82(4), 959-70. Fiskerstrand et al 2010, J Molec Diag, 12, 125-31.

Table 1: Common founder mutations. Figures show only probands (i.e. screening results/confirmations of research screening) and not familial/cascade testing.

* Fin_{major} mutation = c.1408-34_1408-6del splicing mutation in MKS1 intron 15

All mutant individuals had bi-allelic (homozygous or compound het.) mutations. Extensive cascade carrier and confirmation testing has been carried out both in families identified in the research community and directly in our work (see table 3) due to severity of the disorder. Thus far, one prenatal test has been carried out, with one more scheduled.

	MKS1:	TMEM67:
Families submitted for full screening:	13	9
Positives (two confirmed pathogenic mutations):	2	2

Table 2: Full diagnostic screens performed.

A fluorescent sizing PCR has been developed to detect the common MKS1 Fin_{major} mutation (29bp deletion in intron 15) for rapid diagnosis in carrier families (fig.3).

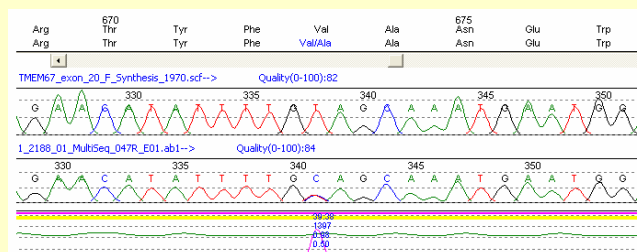


Fig. 2: Heterozygous TMEM67 missense change (c.2018T>C, p.Val673Ala) in mildly affected MKS3 patient & sibling. Inherited in trans with a nonsense mutation.

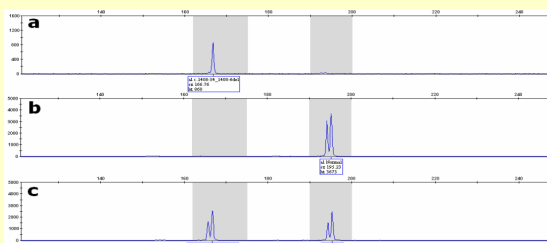


Fig. 3: MKS1 Fin_{major} (c.1408-34_1408-6del) sizing assay results, showing FAM-labelled PCR product peaks for a homozygous mutation (a), homozygous normal (b) and heterozygote (c).

Gene:	Unique Mutations:	No. of families:	Confirmation tests:	Negative carrier tests:	Positive carrier tests:	Prenatal tests:
MKS1 (FLJ20345)	5	4	3	0	6	1 (normal)
TMEM216 (MKS2)	1	1	2	1	1	-
TMEM67 (MKS3)	6	8	7	8	23	-
CEP290 (MKS4)	-	-	-	-	-	-
RPGRIP1L (MKS5)	3	2	2	0	4	-
CC2D2A (MKS6)	1	1	-	-	-	-
NPHP3 (MKS7)	-	-	-	-	-	-

Table 3: Cascade testing in affected families (as of March 2011). "Unique mutations" = number of different mutations tested in gene. "Positive carrier tests" includes parents of affected probands. Hyphens denote no testing.

This service has thus far detected or confirmed pathogenic Meckel syndrome mutations in 16 families. The laboratory is CPA accredited and workflows are in place to develop rapid bespoke testing for any MKS gene mutations not offered under the current routine service (e.g. research confirmations).

Future developments will involve the use of next-generation sequencing to cover more of the MKS (and wider ciliopathy) spectrum.

This service is provided under UKGTN (www.ukgtin.org) and is publicised internationally on GeneTests (www.genetests.org).

For further details see the Yorkshire Regional DNA Laboratory website: <http://www.leedsth.nhs.uk/sites/leedsthdna/>