

Practice Guidelines for Molecular Analysis of Colorectal Polyposis (Familial Adenomatous Polyposis Coli and *MUTYH*-Associated Polyposis)

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These guidelines are currently provisional and consequently subject to change.

1. Nomenclature and gene IDs

OMIM number	Condition	Gene name	Gene map locus	Reference Sequence
175100	Familial adenomatous polyposis	<i>APC</i>	5q21-22	NM_000038.3
135290	Hereditary desmoid disease	<i>APC</i>	5q21-22	NM_000038.3
604933	<i>MUTYH</i> -associated polyposis	<i>MUTYH</i>	1p34.2-32.1	NM_012222.2

Description of diseases

2.1 Familial adenomatous polyposis

Approximately 1-2% of all colorectal cancer can be attributed to familial adenomatous polyposis (FAP) which is an autosomal dominantly inherited syndrome caused by mutations in the adenomatous polyposis coli (*APC*) gene. The main characteristic of FAP is the presence of hundreds to thousands of polyps throughout the colon and rectum which if not detected at an early stage inevitably results in colorectal cancer. The polyps are usually present by the second decade of life, becoming symptomatic by the third decade. In approximately 30% of cases the disease arises *de novo* [1]. A milder form of the disease, attenuated FAP, is characterised by the presence of fewer polyps, typically less than 100 and a later age of both onset of polyps and colorectal cancer [2]. Extra colonic gastrointestinal manifestations include the presence of gastric polyps and small bowel adenomas. Other extra colonic manifestations

include desmoid tumours, epidermoid cysts, osteomas and dental abnormalities [1]. Up to 90% of individuals have retinal lesions termed congenital hypertrophy of the retinal pigment epithelium or CHRPE. More rarely the condition is associated with hepatoblastomas, thyroid tumours, adrenal tumours and brain tumours (Turcot syndrome). Familial infiltrating fibromatosis or desmoid disease is also caused by mutations in the *APC* gene [3]

Genotype/phenotype correlations are well known in FAP. Patients with mutations between codons 168-1580 have a classic polyposis with mutations in the central region of the gene from codons 1290-1400 giving rise to a profuse polyposis with thousands of intestinal polyps [4]. Mutations in the extreme 5' and 3' regions of the gene or in the alternatively spliced region of exon 9 typically have an attenuated phenotype [5, 6, 7]. Extra colonic manifestations are found in association with more 3' mutations and familial desmoid disease is associated with mutations proximal to codon 1400. CHRPE are associated with mutations between codons 457 and 1444 [8]. However inter and intra familial variability is seen which has been explained by modifiers and environmental factors. Somatic mosaicism may also cause a deviation from the expected phenotype and this has been seen in up to 11% of *de novo* cases [9].

Mutations in the *APC* gene can be detected in up to 90% of classical cases of FAP but may only be detectable in 20-30% of attenuated FAP cases [10]. Virtually all mutations causing FAP are truncating mutations, with up to 80% being point mutations and a further 7-12% as large genomic deletions [10,11]. Two missense variants, p.Ile1307Lys and p.Glu1317Gln have been associated with an increased risk of colorectal cancer and were originally considered as cancer predisposing genes [12,13], although this association has more recently been refuted (see below) The p.Ile1307Lys mutation is found at a prevalence of 6% in the Ashkenazi Jewish population [12].

2.2 *MUTYH*-associated polyposis (MAP)

MUTYH-associated polyposis (MAP) is an autosomal recessive condition giving the appearance of being sporadic [14]. It is characterised by multiple adenomas, but generally not the thousands seen in FAP, in the colon. It tends to present around the age of 50 years. These observations, however, are not absolute and there is significant overlap both in polyp number and in age at diagnosis between colorectal polyposis caused by *APC* and *MUTYH* mutations. The majority of MAP individuals with

polyps will go on to develop colorectal cancer and in many, cancer is already present at the time of diagnosis.

MAP is difficult to differentiate clinically from attenuated FAP and family history may be the best indicator of aetiology with dominant transmission suggesting FAP and occurrence of multiple affected sibs to unaffected parents suggesting MAP.

Whilst MAP generally has a similar clinical picture to attenuated FAP, there are reports of possible associations with extracolonic manifestations including endometrial cancer, gastric and duodenal adenomas and breast cancer although published data remain scant [15]

No statistically or clinically significant association has been demonstrated between carrying a single *MUTYH* mutation and increased risk of colorectal cancer [16] and MAP must therefore be considered a truly genetically recessive cancer predisposition condition.

The *MUTYH* gene is a base excision repair gene. Two mutations, p.Tyr176Cys and p.Gly393Asp account for around 82% of mutant alleles in the UK Caucasian population [17]. The p.Tyr101X and p.Glu477X mutations have been found in individuals of Asian origin (the p.Glu477X mutation appears to be specific to Gujaratis[18]) and the mutation c.1428_1430delGGA (p.Glu477del) has been associated with Southern European populations [19]. (NB mutation nomenclature given here may differ from original publications due to an additional 33 nucleotides in the current version of the reference sequence (NM_012222.2, as of January 2009, compared to the earlier version).

3. Referral categories for molecular testing

A diagnosis of FAP can usually be based on the colorectal phenotype plus other extra colonic manifestations and affected individuals are referred for mutation analysis. Attenuated disease is less easily identified clinically as features overlap with MAP (see above) and in some cases where few polyps are present, with HNPCC.

Once a mutation has been identified, at risk relatives are referred for presymptomatic testing. Carrier testing may be offered in MAP families (see below).

Presymptomatic testing of children should only be initiated once they are at an age when bowel screening can be started. The age at which this

occurs may vary from Centre to Centre and may also depend on the clinical features in a family but is usually in the early teens.

4. Testing strategies.

The decision to screen for *APC* or *MUTYH* mutations may vary from Centre to Centre. In many cases this will be a clinical request and the laboratory would not be expected to make that decision themselves.

In other cases, for a new polyposis referral, the laboratory may have its own testing algorithm which may be influenced by phenotype or family history. For example, where there is florid polyposis in the context of a dominant family history, *APC* mutation analysis may be considered the most appropriate starting point. *MUTYH* analysis may be more appropriate when there is an attenuated phenotype with the suggestion of recessive inheritance or for an apparently sporadic case.

Alternatively, a more pragmatic approach may be to test all polyposis index cases first for *MUTYH* mutations p.Tyr176Cys and p.Gly393Asp (and p.Glu477X for Asian individuals) on the basis of common mutation frequency and simplicity of analysis and then full *APC* gene screening for *MUTYH* negative cases.

4.1 *APC* MLPA

Given the relative analytical simplicity of MLPA versus full gene scanning for *APC* mutations and the relative frequency of genomic rearrangements, this may be considered the first step in analysis of a FAP/ colorectal polyposis index case. MLPA kits are available from MRC Holland (kit reference P043) and analysis software packages are freely available as downloads from the MRC Holland website (“Coffalyser”: <http://www.mlpa.com/coffalyser/>) or from the NGRL (Manchester) website (<http://www.ngrl.org.uk/Manchester/Informaticspubs.htm#MLPA>). Alternatively the GeneMarker package from SoftGenetics is widely used for analysis.

It should be noted that in the newest version of the *APC* kit from MRC Holland, the exons have been renumbered to reflect the numbering on the Genbank reference sequences which means that the largest exon which has previously been labelled as exon 15 is now exon 18 in this kit. However until agreement is reached on this issue more widely, it is recommended that the exon containing the A of the first ATG is left as

exon 1. This maintains the numbering system which is most widely known to laboratories and clinicians.

It should be noted that the current commercially available MS-MLPA kits are not certified for diagnostic use and must be fully validated in individual laboratories prior to implementation. We recommend that recurrent variation observed in the MRC MS-MLPA kit is reported to the manufacturer to facilitate future kit development.

4.2 APC mutation analysis

Mutations are spread throughout the whole of the *APC* gene and many hundreds have now been identified [8 20, 21]. Clinical details can be used to narrow the region of the gene to be analysed though in practice with high throughput analysis in place this is now less useful. However in patients with attenuated disease, initial screening of the 5' and 3' portions of the gene plus the alternative spliced exon, exon 9, may be considered. Point mutations in *APC* have been identified both by direct sequencing and by a variety of mutation scanning techniques such as SSCP, DGGE, dHPLC, and CSCE. In each of these techniques exons 1-14 and overlapping segments of exon 15 are analysed separately.

The protein truncation test (PTT) remains a useful alternative method to screen for truncating mutations [22]. Exon 15 can be analysed in four overlapping sections directly from genomic DNA. Approximately 66% of *APC* mutations are located in exon 15 and can therefore be detected by PTT. Exons 1-14 can be analysed in a single step but requires RNA as starting material and there are more problems, in part due to alternate splicing.

The two most common mutations, 5bp deletions: c.3183-3187delACAAA, p.Gln1062X (historically referred to as the codon 1061deletion) and c.3927_3931delAAAGA, p.Glu1309AspfsX4 (the codon 1309 deletion), account for around 15-20% of cases can also be analysed directly by PCR followed by either polyacrylamide gel or Nusieve gel electrophoresis. Probes detecting both these deletions are included in the MRC Holland MLPA kit P043. Detection of either deletion by MLPA should be confirmed by an alternative method such as gel electrophoresis or sequencing to exclude the (unlikely) possibility of a SNP under the MLPA probe hybridisation site.

4.3 Linked marker analysis

A number of well characterised polymorphic microsatellites have been used in the past as linked markers for presymptomatic testing in FAP families in which no *APC* mutation can be identified. This strategy must now be used with caution because of the phenotypic overlap between FAP and *MUTYH*-associated polyposis. Linked marker analysis may be considered appropriate when no *APC* mutation has been identified following exhaustive analysis of *APC* (including MLPA) and where there is a strong *dominant* family history of colorectal polyposis.

At least 5 intragenic RFLP's have been identified. The error due to recombination for these markers is negligible. In the majority of families, the intragenic markers are in linkage disequilibrium. There are a few families informative for one marker and uninformative with another. Microsatellite markers closely flanking the *APC* gene are also available.

In the majority of families, it should be possible to obtain informative results for at least one proximal and one distal marker using the microsatellite markers listed below. In families with no living affected individuals it is sometimes possible to obtain paraffin blocks of normal tissue from which DNA can be extracted. Tumour tissue DNA can give misleading results in linked marker analysis due to loss of heterozygosity in the region of the *APC* gene. PCR analysis of fragments up to around 250bp seem to work well, larger fragments, less so.

Primer details are given in Tables 1 and 2

4.4 Cytogenetic Analysis

De novo and inherited deletions and translocations disrupting the *APC* gene detectable cytogenetically have been identified in a small number of individuals/families. Chromosome investigations may be worth carrying out if no mutations have been found using the techniques listed above.

4.5 *MUTYH* common mutations

MUTYH mutations p.Tyr176Cys and p.Gly393Asp (and p.Glu477X) are single nucleotide substitutions amenable to analysis using a variety of well established techniques.

5. Interpretation of results

5.1 *APC* mutations

5.1.1 Diagnostic testing

Most clearly pathogenic *APC* mutations are those which disrupt *APC* gene structure (deletions or other genomic rearrangements detected by MLPA) or predicted *APC* protein structure (truncating mutations – nonsense, frameshift or splice site mutations). These mutations can be reported as causative and confirm a diagnosis of FAP. Presymptomatic testing can then be offered to relatives at risk of the disease following appropriate genetic counselling.

If a mutation is not identified, the report should state the extent of the analysis and also include the expected detection rate. The mutation detection rate using sequencing is up to 90% in typical FAP patients. Duplications or deletions detectable by MLPA account for 8-12% of all mutations.

There are few examples of pathogenic missense variants and caution must be exercised in reporting such changes. Both the p.Ile1307Lys and the p.Glu1317Gln variants have been associated with an increased predisposition to colorectal cancer [12,13] although the literature on both variants is confusing and conflicting. The risk of colorectal cancer associated with p.Ile1307Lys has been reported to be as high as 10-20% [12] and colonoscopic surveillance for p.Ile1307Lys carriers has been suggested [23]. However, other studies have found no statistically or clinically significant link between colorectal cancer risk or phenotype and p.Ile1307Lys and do not recommend further surveillance [24, 25, 26, 27]. Similarly, earlier reports of increased colorectal cancer susceptibility in carriers of *APC* p.Glu1317Gln [13] have not been substantiated and this variant is considered to be not clinically significant [28, 29]. Detection of either of these variants in a routine screen should be commented on but they are not considered to be associated with classic FAP and therefore cannot be interpreted as confirming a diagnosis. Predictive testing for relatives of carriers of either of these variants is inappropriate and should not be offered.

A suggested form of words for reporting either of these variants is:

“(this patient) was found to carry the APC p.Ile1307Lys/p.Glu1317Gln variant. No other variants were detected. This variant was formerly considered a predisposition allele for colorectal cancer, however, more recent papers [cite ref(s)] indicate that there is no statistically or clinically significant association between carrying the variant and

increased risk of colorectal cancer. Detection of this variant does not confirm a diagnosis of FAP and predictive testing for this variant is not indicated in (this patient's) relatives.”

A small number of missense mutations (and synonymous nucleotide changes) have been reported where pathogenicity is due to their proximity to splice sites and disruption of correct splicing rather than the consequences of the amino acid substitution on APC protein structure and function *per-se* [30, 31]. Other missense mutations are of unknown significance and should be evaluated and reported accordingly.

5.2.2 Predictive testing

A positive result means that a patient is highly likely to develop FAP. Given that *APC* mutations are almost 100% penetrant, “highly likely” can be qualified further as almost certain to develop FAP. Absence of the familial mutation means that the individual is highly unlikely to develop FAP but reports should indicate that they remain at population risk of sporadic colorectal cancer.

5.2.3 Prenatal testing and preimplantation genetic diagnosis (PGD)

Prenatal testing is rarely requested for FAP but has been carried out on a number of occasions. Similarly PGD has also rarely been carried out for this condition.

5.1 *MUTYH* common mutations

5.1.1 Diagnostic testing

The presence of two of the common *MUTYH* mutations confirms a diagnosis of MAP. The presence of one mutation in an individual with colorectal polyposis and a pedigree consistent with autosomal recessive inheritance may indicate the presence of a rare mutation elsewhere in *MUTYH* but may also be coincidental. Full *MUTYH* gene mutation analysis for rare mutations would be indicated in such.

5.1.2 Carrier and predictive testing

Once *MUTYH* mutations have been identified, carrier testing may be offered to the partner of an affected individual in order to assess genetic risk to any offspring. In this circumstance, it is reasonable to just test for

just the p.Tyr176Cys and p.Gly393Asp mutations (and p.Glu477X in Asian families).

If the partner of an affected individual is revealed to be a carrier, any offspring will be at 50% risk of MAP and predictive testing should be offered. Predictive testing of offspring is currently not indicated if the unaffected partner does not carry one of the mutations tested for.

Predictive testing should be offered to any sibling of an affected individual who will be at 25% risk of MAP.

Table 1: Intragenic markers

Location	Restriction digest	GDB ID	Reference
5' UTR	Dde1		
Exon 11	Rsa1	186686	
Exon 15	Msp1		
	BSAJ1		
3'UTR	Ssp1	186862	

Table 2: Linked markers

Microsatellite	Locus	Location	Error	PIC	Size	GDB Id
CB26	D5S299	Proximal	8%	0.66	156-182	185754
YN5.64	D5S82	Proximal	4%	0.70	169-179	180445
CB83	D5S122	Proximal	2%	0.19	211,213	180444
LNS	D5S346	Distal	<1%	0.83	96-106	181171
MBC	MCC gene	Distal	<1%	0.49	168-176	181466
CA25	D5S318	Distal	5%	0.78	116-128	186851

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