

Best practice guidelines for molecular analysis of colorectal polyposis: familial adenomatous polyposis coli (FAP) and *MUTYH*-associated polyposis (MAP)

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Abstract

Background: UK Clinical Molecular Genetics Society (CMGS) consensus best practice guidelines for molecular analysis of familial adenomatous polyposis coli (FAP) were published in 2000. Technological developments in molecular testing for FAP together with the clinical and molecular characterisation of *MUTYH*-associated polyposis (MAP) led to the need to update the original FAP guidelines which were “retired” in December 2007. This update presents consensus best practice in the molecular analysis of clinically related colorectal polyposis syndromes of FAP and MAP.

Methods: Testing and reporting guidelines have been drawn up and agreed in accordance with the procedures of the UK Clinical Molecular Genetics Society.

Results: A practical set of molecular genetic testing and reporting guidelines has been developed for both familial adenomatous polyposis coli and *MUTYH*-associated polyposis. In addition, advice is given on appropriate reporting policies, including advice on test sensitivity and recurrence risks.

Conclusion: An agreed set of practice guidelines has been developed for the diagnostic molecular genetic testing of colorectal polyposis.

Background

Colorectal polyposis

Colorectal cancer due to hereditary syndromes can be divided into two distinct categories: polyposis and non-polyposis. This article summarises current practice in the molecular genetic analysis of colorectal polyposis. Gene nomenclature and accession references are given in table 1.

Familial adenomatous polyposis

Less than 1% of all colorectal cancer can be attributed to familial adenomatous polyposis (FAP) and in countries with established registries and prophylactic surgery this figure is falling. FAP is an autosomal dominantly inherited syndrome caused by mutations in the adenomatous polyposis coli (*APC*) gene. The main characteristic of the disease 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 at a later age of 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 affected 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 generally have a classic polyposis, and patients with mutations in the central region of the gene (codons 1290-1400) have a profuse polyposis with thousands of intestinal polyps [4]. Patients with 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 mutations at the 3' end of the gene and familial desmoid disease is associated with mutations proximal to codon 1400. CHRPE is associated with mutations between codons 457 and 1444 [8]. Inter- and intra-familial variability is seen which can be explained by modifiers and environmental factors and somatic mosaicism may also cause a deviation from the expected phenotype. Mosaicism 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% being 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 mutations [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].

***MUTYH*-associated polyposis (MAP)**

MUTYH-associated polyposis (MAP) is an autosomal recessive condition [14]. It is characterised by multiple adenomas in the colon, but generally not the thousands seen in FAP. 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.

There is no consistent evidence that the presence of a single *MUTYH* mutation is associated with multiple colorectal polyposis and therefore MAP must be considered a truly recessive polyposis syndrome. There is evidence that obligate monoallelic *MUTYH* mutation carriers (first degree relatives of biallelic MAP patients) have a modest increased incidence of colorectal cancer compared with the general population although consensus surveillance guidelines for this cohort remain to be developed.

MAP is difficult to differentiate clinically from attenuated FAP and family history may therefore 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]. There has also been an association with sebaceous adenoma giving overlap with Muir Torre syndrome [39]

The *MUTYH* gene is a base excision repair gene. Two common mutations, p.Tyr179Cys and p.Gly396Asp account for around 82% of mutant alleles in the UK Caucasian population [17]. The p.Tyr104X and p.Glu480X mutations have been found in individuals of Asian origin (the p.Glu480X mutation appears to be specific to Gujuratis [18]) and the mutation c.1437_1439delGGA (p.Glu480del) has been associated with Southern European populations [19].

Methods

Current practice in the molecular analysis and reporting of colorectal polyposis was assessed by consideration of the external quality assessment returns submitted to the United Kingdom External Quality Assessment Scheme (UKNEQAS) over a five year period. These guidelines were posted on the web-site of the UK Clinical Molecular Genetics Society (CMGS) for consultation and amendment between 1st September, 2009 and 6th May, 2010 and heads of the constituent laboratories were invited to comment. In the light of feedback amendments were made and the final document was ratified by the CMGS Executive Committee on 20th May, 2010.

Results

Gene Nomenclature

Please note that *MUTYH* mutation nomenclature given throughout these guidelines may differ from published literature because of the use of different reference sequences (see table 1). All mutation nomenclature must follow the recommendations of the Human Genome Variation Society (HGVS) mutation nomenclature guidelines (<http://www.hgvs.org/mutnomen/>).

There are five known isoforms of the *MUTYH* protein which result from three alternative splice acceptor sites at the intron 2/exon 3 boundary and two alternative ATG translation initiation codons. The original reports describing the association between *MUTYH* mutations and polyposis [14, 17] were based on reference sequence NM_001048171, the transcript for protein isoform 2. Using this reference sequence, the common Caucasian mutations were designated p.Tyr165Cys and p.Gly382Asp. In accordance with HGVS guidelines, it is recommended that clinical reports describe variant nucleotide and amino acid numbering relative to the longest isoform (transcript reference sequence NM_001128425.1; isoform 5).

Referral categories for molecular testing

A diagnosis of FAP can usually be made 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 Lynch syndrome (hereditary non-polyposis cancer).

Once a mutation has been identified, at risk relatives are referred for presymptomatic testing and 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 but may be as young as 10.

Testing strategies.

Testing strategies may vary between centres and in most cases prioritisation of cases will be determined by the referring clinician. For a new polyposis referral a pragmatic testing strategy would be to test first for *MUTYH* common mutations; p.Tyr179Cys and p.Gly396Asp (and p.Glu480X for Gujarati individuals), and then full *APC* gene screening for *MUTYH* negative cases. Alternatively testing strategies 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 or where there are the typical extracolonic features of Gardner syndrome. *MUTYH* analysis may be more appropriate when there is an attenuated phenotype with the suggestion of recessive inheritance or for an apparently sporadic case.

Testing Methods

Multiplex ligation-dependent probe amplification (MLPA): MLPA is now routinely used for the simultaneous assessment of gene dosage and this method is used to detect exonic deletions and duplications within the *APC* gene. 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 a reasonable first step in the analysis of a FAP/colorectal polyposis index case. MLPA kits are available from MRC Holland and analysis software packages are freely available as downloads from the MRC Holland website (“Coffalyser”: <http://www.mlpa.com/coffalyser/>) or from the National Genetics Reference Laboratory (Manchester) website (<http://www.ngrl.org.uk/Manchester/Informaticspubs.htm#MLPA>). Alternatively the GeneMarker package from SoftGenetics is widely used for analysis. MRC-Holland have recently introduced a MLPA kit for *MUTYH* exon dosage analysis although data on pathogenic *MUTYH* rearrangements remain scant.

It should be noted that in the newest version of the *APC* kit from MRC Holland (kit reference P043) 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 given as exon 18. However until agreement is reached on this issue more widely, it is recommended that the exon containing the A of the first ATG is numbered from 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 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 any MRC-Holland MLPA kit is reported to the manufacturer to facilitate future kit development.

Other methods for *APC* exon dosage analysis such as exon-specific qfPCR, linked SNP or microsatellite analysis, karyotyping and/or cytogenetic FISH analysis, Southern analysis with cDNA probes or array CGH may be considered in depending upon the resources and expertise of the laboratory but these are not in common usage.

Point mutation analysis: 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. 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 to exclude the (unlikely) possibility of a SNP under the MLPA probe hybridisation site.

Point mutations in *APC* can be successfully identified by either direct sequencing or a range of mutation scanning techniques, in each of these, exons 1-14 and overlapping segments of exon 15 are typically analysed separately. 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 rarely practiced. However in patients with attenuated disease, initial screening of the 5' and 3' portions of the gene plus the alternatively spliced exon 9, may be considered. A locus specific database for *APC* can be found at chromium.liacs.nl/LOVD2/colon_cancer and this site also gives two further external links to additional databases.

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 however this approach is problematic in part due to alternate splicing of the *APC* gene.

MUTYH mutations p.Tyr179Cys and p.Gly396Asp (and p.Glu480X) are single nucleotide substitutions amenable to analysis using a variety of well established techniques. Sequencing of the full *MUTYH* gene coding sequence may be considered depending upon clinical and ethnic considerations.

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 be used with extreme caution because of the phenotypic overlap between FAP and MAP. 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 restriction fragment length polymorphisms have been identified. The error due to recombination for these markers is negligible. There are a few families informative for one marker and uninformative with another however for the majority of families, the intragenic markers are in linkage disequilibrium. 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. In families with no living affected individuals it is sometimes possible to obtain paraffin blocks of normal tissue from which DNA can be extracted. However it is important to note that tumour tissue DNA can give misleading results in linked marker analysis due to loss of heterozygosity in the region of the *APC* gene (F MacDonald personal communication).

Primer details are given in Tables 2 and 3.

Cytogenetic Analysis: *De novo* and inherited deletions and translocations disrupting the *APC* gene which are detectable cytogenetically have been identified in a small number of individuals/families. Chromosome investigations may be considered if no mutations have been found using the techniques given above.

Interpretation of results

***APC* mutations**

Diagnostic testing: Most clearly pathogenic *APC* mutations are either deletions or other genomic rearrangements detected by MLPA or truncating mutations – either 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. Of these duplications or deletions detectable by MLPA account for 8-12% of all mutations.

There are few examples of definite pathogenic missense mutations 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 pathogenic missense variants (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.

Predictive testing: The presence of a pathogenic mutation 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.

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.

***MUTYH* mutations**

Diagnostic testing: The presence of two *MUTYH* mutations confirms a diagnosis of MAP. The presence of one common 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 sequence analysis of the *MUTYH* coding sequence and splice sites and MLPA would be indicated in such a case. Full testing would also be indicated in affected siblings with consanguinous parents without polyposis.

Carrier and predictive testing: Once *MUTYH* mutations have been identified in a family member, carrier testing may be offered to the partner of an affected individual in order to assess genetic risk to any offspring. Carrier testing of partners may be limited testing to the p.Tyr179Cys and p.Gly396Asp mutations (and p.Glu480X in Gujarati families). Alternatively exclusion of a rare mutation by analysis of the full coding sequence and MLPA will virtually exclude the risk of MAP to any offspring.

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.

Discussion/conclusion

A practical set of guidelines has been developed for laboratories undertaking the molecular genetic analysis of colorectal polyposis. Feedback has been obtained from the constituent laboratories of the CMGS (46 laboratories from the UK and Ireland). All comments received were minor; largely typographic corrections and some points of clarity. There was no disagreement on the recommendations made. All comments have been incorporated into this final document.

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Table 1: Nomenclature and gene accession references.

OMIM number	Condition	Gene name	Gene map locus	cDNA 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_001128425.1

Table 2: *APC* intragenic markers

Location	Restriction digest	Reference
5' untranslated region	DdeI	32
Exon 11	RsaI	33
Exon 15	MspI	34
Exon 15	<i>Bsa</i> JI	35
3'untranslated region	SspI	36

Table 3: Linked markers (37, 38)

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	<i>MCC</i> gene	Distal	<1%	0.49	168-176	181466
CA25	D5S318	Distal	5%	0.78	116-128	186851

I have some questions about this table:

Is the title enough – could we replace it with something like:

“Table 3: Microsatellite markers used for *APC* gene tracking (adapted from references 37 and 38).”

Does “*MCC* gene” need a bit more explanation? offspring

Can we just say “error” or does this need qualifying – would “recombination error” be better or can you think of a better way of saying it?

Do we need to give PIC in full?

Is “size” an important consideration for a review like this

Is GDB ID self explanatory or is it an abbreviation that needs qualifying?

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