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How can histopathologists help clinical genetics in the investigation of suspected hereditary gastrointestinal cancer?

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Abstract

Histopathologists are critical in the diagnosis of hereditary gastrointestinal tumours. This is increasingly important as genetic testing becomes more available and the benefits of anti-cancer surveillance in those at increased risk are realised. Cancer genetics services should include pathologists and be organised on multidisciplinary team lines. Hereditary cancer syndromes predispose to tumours throughout the GI tract. Lynch syndrome is the most prevalent hereditary GI cancer condition, responsible for ~3.3% of all colorectal as well as other GI and extra-intestinal cancers. Tumour tests to diagnose Lynch syndrome are important in guiding genetic testing, and can be used systematically to screen cancers for the condition. Familial adenomatous polyposis and all the other forms of hereditary polyposis put together account for <1% of all colorectal cancer. However, the histological distinction of the various polyposes, including type, site and numbers of polyps is crucial in informing genetic testing.

Key words

Genetic Predisposition to Disease

Colon

Rectum

Adenocarcinoma

Adenoma

Polyps

Gastrointestinal neoplasms

Biopsy

DNA mismatch repair

Immunohistochemistry

Microsatellite instability

Quality control

Lynch syndrome

Adenomatous polyposis coli

Peutz-Jeghers Syndrome

Juvenile Polyposis Syndrome

Hereditary Mixed Polyposis Syndrome

Cowden Syndrome

Hamartoma Syndrome, multiple

1. Context

A fundamental part of the clinical and laboratory examination of a patient with a gastrointestinal tumour is the histopathology. It is absolutely critical in achieving the diagnosis. This is especially the case where that diagnosis includes a possible hereditary predisposition, because this has implications for both the patient, the relatives, and unrelated individuals who happen to have the same underlying genetic mutation.

Phenotype is defined as "*the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment*" and, therefore, is what you are: a function of your genes and the environment they find themselves in. Histopathology is therefore crucial in defining an individual tumour phenotype, informing in turn genetic investigations and the interpretation of genetic variation found in that patient. The genome, an individual's complete DNA sequence, is now accessible. About one quarter of all the 21,000 or so human genes have an associated clinical phenotype. The protein-coding parts of all genes, constituting about 2% of the genome, is called the exome, and clinical testing of the exome is already available. Hence, as medicine moves from a genetic, single gene, era into the genomic era, so it becomes increasingly important to define phenotype. Dysmorphism, that is abnormalities in gross morphologic development, is an important feature of many genetic conditions and why clinical geneticists are highly trained in recognising such features. While some cancer genetic conditions certainly do have associated dysmorphic features, the histopathologist can be thought of as the cancer geneticist's dysmorphologist – histopathologists having the necessary expertise, skills and tools to define abnormalities in tumours at the macroscopic, microscopic and molecular level, working where necessary with colleagues in related disciplines.

Cancer genetics now accounts for more than half of all the clinical genetics care provided in the UK, and the role of histopathology in the provision of such services is thus proportionately important. We would strongly urge colleagues in genetics, both clinical and laboratory, to work closely with histopathologists, and vice versa: the model of the multidisciplinary team will be familiar as best practice. The importance of critically utilizing key information in pathology reports

is stressed to those who attend e.g. the UK national cancer genetics courses for doctors, counselors and laboratory scientists, as is asking for histopathological review of multiple samples from family members in complex cases. However, whilst the mainstay of clinical cancer genetics has classically been the 'family history', it is becoming apparent that laboratory tests on tumours have better specificity and sensitivity than family history at finding those who harbour inherited mutations. So, as such tests become cheaper to perform, the identification of patients with hereditary predisposition is moving towards systematic screening for biomarkers in incident cases.

Cancer genetic conditions generally predispose to tumours of various types and at more than one site. Whilst histopathologists will usually be presented with tissue from a specific organ, we have decided to take a condition rather than organ-based approach and concentrate on those conditions which are relatively common or warrant careful differentiation on histological grounds.

It should be borne in mind that many syndromes are multi-system and predispose to more than just GI tumours. Also, that this review is not exhaustive and does not need to be, as nowadays there are many online sources of information regarding hereditary cancer, such as the family cancer database (FaCD: http://www.familialcancerdatabase.nl/). Here, the features of known syndromes may be browsed, help can be given with identifying a syndrome from tumour types and symptoms, or searches on a word or gene can be performed. We recommend that the reader refer to the relevant sections of this resource in conjunction with this review. Online Mendelian Inheritance in Man (OMIM; http://www.omim.org/) is another valuable resource: for each gene and phenotype there are OMIM entries. The *Oxford Desk Reference: Clinical Genetics* has sections on GI cancer and associated syndromes.[1]

2. GI-cancer associated genetic conditions

These can be divided into polyposes, in which an excess of polyps is seen, and those in which there is no obvious excess, such as Lynch syndrome and hereditary diffuse gastric cancer.

2.1 Lynch Syndrome

Lynch syndrome (LS) is the most prevalent single-gene disorder predisposing to colorectal cancer. It is caused by constitutional mutations affecting one of four DNA mismatch repair (MMR) genes, specifically *MSH2, MLH1, MSH6* or *PMS2*. In Denmark, where systematic testing for LS is now carried out on all CRC, approximately 3.3% are now known to be due to LS, implying a prevalence of at least 1:500. The main cancers LS predisposes to are CRC and endometrial cancer, but a wide spectrum of associated tumours is now recognised, including from the GI tract, stomach, small bowel, hepatobiliary tract and, possibly, pancreatic cancer, but also upper urinary tract, ovaries, brain, prostate and breast.[2] The average age of onset of CRC is approximately 42y, but varies with the underlying gene, as does the spectrum of associated cancers. An individual with LS has a high, but not inevitable risk of cancer. Current best estimates of risk in mutation carriers to age 70y of any LS-associated cancer are ~60% in men and ~70% in women. Both *MSH2* and *MLH1* are associated with higher risks and younger onset, with *MSH6* and *PMS2* conferring lower risks and at older age, while *MSH6* confers a relatively greater risk of endometrial cancer.[2,3](Table 1)

Table 1. Major cancer risks in Lynch syndrome, by underlying gene.

In the case of *MSH2* there is an additional mechanism of disease in that large deletions in an adjacent gene, *EPCAM* (whose product is expressed in gut mucosal brush border) can affect *MSH2* expression in one of two ways. With deletions encompassing both *EPCAM* and *MSH2* the associated phenotype is indistinguishable from mutations of any sort involving *MSH2* alone. However, deletions only involving *EPCAM* can lead to read through of mRNA into *MSH2*, resulting in expression of a non-functional protein and methylation of the *MSH2* promoter, which prevents any normal MSH2 being produced. Curiously, the associated phenotype is of both large and small

bowel cancers, but not endometrial cancer, which may reflect the tissue expression pattern of EPCAM.

However, because of the tendency in LS to earlier onset and somewhat more survivable cancers, some individuals can and often do develop two or more cancers. The variety of associated tumour types also means that a possible diagnosis of LS may not be immediately obvious to clinicians (or patients) either in an individual or family. Hence, it is important for the pathologist to be aware of such possible combinations and alert colleagues to them, if necessary after appropriate investigation and discussion within an MDT that includes cancer genetics input.

The cells of individuals with LS have proficient DNA mismatch repair (MMR), but if a somatic mutation occurs in the other copy of the respective gene then MMR deficiency results in that cell. One consequence of this is that the genome of such cells starts to accumulate innumerable small mutations, particularly, but by no means exclusively in repetitive stretches of DNA, called microsatellites, resulting in the phenomenon of microsatellite instability (MSI). Another is that the loss of MMR function is manifest as abnormal or lost expression of the affected MMR protein (and often its binding partner), readily detectable by immunohistochemistry (IHC). Concomitantly, and probably because MMR is necessary for chromosomal recombination to occur, such cells then stop accumulating large scale chromosomal defects, classically manifest as chromosomal instability, classically typified by loss of heterozygosity (LOH). Hence, tumours which have lost MMR not only have MSI and abnormal MMR expression, but are typically near-diploid.

It has been suggested that the raised mutation rate in MMR deficient cells is the Darwinian selectable advantage driving such tumours. However, while the phenomenon of MSI is certainly diagnostically useful there is good reason to believe that it may just be a paraphenomenon, albeit diagnostically useful: that the mutation rate in cells is not limiting and that it is a reduction in MMR-triggered apotosis which is the driver. It is also suggested that the CRC in LS develop much faster than in the general population, with the high frequency of interval cancers despite frequent (2 yearly) colonoscopy being cited as evidence. However, this presupposes that such cancers all

arise in adenomas and the role of the serrated lesion in LS may be more significant than has been previously recognised.

2.1.1 *Muir-Torre syndrome and LS*

Muir-Torre syndrome (MTS) is the occurrence in the same individual of keratoacanthomas or sebaceous adenomas/carcinomas with an internal cancer and is common in LS, more often, but not exclusively associated with mutations in *MSH2*. However, LS is probably not the only cause of MTS.

2.1.2 *Colorectal cancer pathology in LS*

Colorectal cancers (CRC) in LS have a skewed anatomical distribution, being more likely to occur in the colon than rectum compared to sporadic CRC. It is important to understand, however, that approximately 12% of colon cancers have sporadic loss of MMR and hence MSI, but MSI in younger CRC is more likely due to LS. Hence, most MSI in colon cancers is sporadic, but this proportion is highly age dependent: about 25% of CRC at 35y are due to LS, reducing to 4% at 55y and 3% ≥60y, whereas the rate of sporadic MSI is 2% between 35-55y, then rises to 12% at 70y.[4]

LS CRCs are more likely to have mucinous or poorly differentiated histology, exhibit high grade (poor) differentiation, and have tumour infiltrating lymphocytes, but these features are by no means absolute.[5] In particular, while in the past it has been commented that CRCs in LS show a marked peritumoural Crohns-like reaction of lymphoid follicles, it actually turns out that this is more common in CRC without MSI.[6] For a given stage, however, LS CRC do appear to have improved survival, for reasons that may include better response to chemotherapy, different host immune response, younger age of the patient and reduced propensity to metastasize, but exactly why remains unclear.

Adenomas in LS tend to be larger, villous, with high grade dysplasia and undoubtedly cancers arise in them. A smaller proportion of adenomas than carcinomas are found to have lost MMR in LS, but it is higher than in the general population, so the significance of finding it in an adenoma is

greater. It is variable as to when in an individual tumour's development that it loses MMR function, and it is not necessarily at the initiation of the lesion. However, there are a number of lines of evidence suggesting that serrated lesions, such as sessile serrated lesions/polyps/adenomas and serrated adenomas, play a greater role in the pathogenesis of CRC in LS than hitherto suspected. Firstly, It has been found that in the large bowel mucosa of individuals with LS about one crypt per cm^2 has deficient MMR.[7] So, adult LS patients have hundreds of aberrant crypts, but they only develop a modest number of CRC, if any, which does not fit with the idea of rapid and inevitable progression of adenomas into carcinomas in LS. Secondly, the reduction in mortality consequent upon intensive colonoscopic surveillance in LS is a function of downstaging rather than adenoma removal: approximately 70% of the CRC detected in LS patients undergoing colonoscopy are Dukes' A, compared to about 17% in LS patients not undergoing surveillance.[8] Thirdly, serrated lesions are now known to give rise to CRC with MSI. And, fourthly, the CaPP2 trial of aspirin chemoprophylaxis in LS showed no reduction in the numbers of adenomas in those on the drug whereas a very significant reduction in CRCs was seen several years after cessation of aspirin treatment, suggesting that whatever premalignant lesion aspirin is acting upon takes time to develop into a cancer and may not be adenomatous.

2.1.3 *Tumour tests in LS: MSI*

Microsatellites are stretches of short repeat sequences in DNA. In a cell in which MMR is lost such repeats undergo contraction or expansion mutations, which are evident when they are compared to the normal constitutional DNA of the individual. Originally, dinucleotide repeats, e.g. $(CA)_n$ were used, but it has since become evident that mononucleotide repeats, e.g. $(A)_n$ or "polyA" are more sensitive markers. Instability at a single microsatellite does not necessarily confer a diagnosis of microsatellite instability (MSI), usually this is dependent on finding instability at, say, 2 out of 5 markers. However, it has to be borne in mind that the markers in common use were originally used to find MSI in colon cancers, and when looking at cancers at other sites, including e.g. rectum, endometrium etc., they are less sensitive. They are also less sensitive in benign tumours, e.g. colorectal adenomas, and less sensitive when the underlying genetic defect is in *MSH6* or

PMS2. Sensitivity has also been shown to depend on the proportion of tumour DNA being tested. Laser microdissection is not feasible for routine diagnostics, but macrodissection definitely improves test performance. If endeavouring to get a result with a small biopsy, immunohistochemistry is the better option.[9]

Specificity is a major issue, as a proportion of all sporadic tumours show MSI. In the colon about 12% of adenocarcinomas show MSI, of which about 1-in-4 will be due to LS, the rest sporadic. In contrast, rectal cancers rarely exhibit MSI, but when they do it usually indicates LS.[10] A similar proportion of gastric and other GI adenocarcinomas also exhibit MSI, so finding MSI is not in itself sufficient evidence of LS, rather the whole setting of age of onset, tumour type, how other relatives may have been affected, all need to be taken into consideration. Hence, the strong recommendation for MDT working in which genetics input is included. While finding MSI does not necessarily equate to LS, not finding MSI, otherwise known as microsatellite stability (MSS) is good to excellent evidence that LS did not cause that tumour, but it has to be considered that some tumours, e.g. due to *MSH6* may not show MSI in >1 of 5 markers (so MSI-L, see below) and Lynch patients will occasionally suffer from sporadic tumours. Lastly, the MSI typically seen in LS cancers is called MSI-high (MSI-H), where a good proportion of markers are unstable, in contrast to MSI-low (MSI-L) which is instability in around 1 of 5 markers: MSI-L, as described in sporadic tumours often of serrated lineage.

2.1.4 *Tumour tests in LS: BRAF & MLH1 promoter methylation*

As a way to determine if a colon cancer with MSI is sporadic, it is possible to test for the presence of a specific oncogene mutation in tumour DNA: *BRAF* p.Val600Glu or “V600E”. If such a mutation is present then the tumour is sporadic in origin. However, while LS colon cancers do not acquire this mutation, a proportion of sporadic tumours do not either. Early estimates were that ~15% of sporadic colon cancers with MSI did not have *BRAF* V600E, but the true proportion is looking to be lower, perhaps 5%. The significance of *BRAF* V600E in tumours at other sites is less clear. In endometrial cancers, for example, it is of no diagnostic use. Recently, detection of *BRAF* V600E has been described using IHC, although its reliability has been questioned.[11]

Sporadic colon cancers with MSI have usually, but not invariably, lost MMR because of hypermethylation of the promoter of *MLH1* on both chromosomes turning off gene expression, often as part of a more general cancer genome methylation phenomenon. It is possible to test for methylation of *MLH1* and it theoretically addresses the issue of lack of specificity of the *BRAF* test. However, a small proportion, perhaps 1% of patients with a CRC with MSI harbour constitutional methylation of *MLH1*, giving them an unusual form of LS and cancers that appear to be sporadic, but are clearly not when their constitutional DNA is compared with that from their tumour. Most such patients with this type of so-called epimutation are not at risk of passing it on to offspring, however, it has been found that in a small proportion there is a risk of transmission. This may be related to chromosomal rearrangements involving the *MLH1* region causing secondary methylation, not unlike the large mutations involving *EPCAM* which cause methylation and so lack of expression of *MSH2*. Hence, this only goes to show that no test is 100% sensitive or specific, and all possibilities must be borne in mind as part of an MDT discussion.

2.1.5 *Tumour tests in LS: MMR immunohistochemistry*

Mismatch repair immunohistochemistry (MMR IHC) is often performed on any suspected LS tumour, including biopsies.[9] It will be appreciated that the technical performance of MMR IHC can vary widely depending on fixation, antigen retrieval, primary antibody and staining platform. It is therefore most important that laboratories participate in EQA schemes such as UK NEQAS ICC (http://www.uknegasicc.ucl.ac.uk/) or Nordic Quality Control (NordiQC; http://www.nordiqc.org/). UK NEQAS ICC has issued guidelines.[12] A good gradient of expression should be demonstrated in crypts, fading out towards the lumen, and stromal / lymphoid cells should be positively stained.(Fig. 1) An important factor in achieving good results is to use normal colon or appendix as controls and not tonsil, in the lymphoid follicles of which MMR proteins are generally expressed at a higher level than in crypts. Hence, adequate staining density in tonsil may mean that mucosal staining is weak. In addition, all efforts should be made to eliminate inappropriate and non-specific staining.

Fig. 1 Normal appendix

Tumour cells will have either stronger expression (DNA repair is upregulated in cells with increased turnover) or abnormal expression. Usually such abnormal expression is manifest as loss of expression, but it can also take more subtle forms, such as loss of nuclear, but gain of cytoplasmic staining.(Fig. 2) In a small proportion of LS tumours, expression may be patchy / weak and not uniform within a tumour. However, MMR protein IHC is particularly fixation-dependent as poorly fixed tumours (a common problem for CRC) show a lack of MMR immunostaining in tumour cells and stromal / lymphoid cells, often affecting all four MMR proteins similarly. To decide whether the patchy / weak staining pattern is a fixation artefact or true biology it helps greatly to have stained for all four MMR proteins, MSH2, MSH6, MLH1 and PMS2. If all show patchy expression, in both tumour and stromal / lymphoid cells, then fixation artefact is likely.

Fig. 2 Colorectal cancers showing loss of MLH1 and MSH2

It is also valuable to test all four markers because of the clues this can give to the underlying genetic defect. MSH2 and MSH6 proteins work as a heterodimer, mutSα, to detect a mismatch in DNA and then recruit the heterodimer mutLα, composed of MLH1 and PMS2, to resolve the defect. So, with an underlying mutation in the *MSH2* gene, MSH2 protein expression is commonly lost together with MSH6. However, MSH6 expression can be retained or abnormal and show shifts from the nuclear to cytoplasmic compartments. Similarly, mutations in *MSH6* typically cause abnormality of MSH6 with retained (or slightly reduced) expression of MSH2. However, a missense mutation has recently been described in *MSH6*, involving its MSH2 binding site, and is thus associated with loss of MSH2 but retention of MSH6 expression in tumours, so this relationship of abnormal expression always being directly related to the underlying genetic defect is not hard and fast. Similarly, loss of MLH1 expression due to underlying *MLH1* mutation (or epimutation/methylation) is often associated with loss of PMS2 expression, but loss of this

combination is rather less often associated with an underlying *PMS2* mutation. In patients with loss of PMS2 expression alone, however, it is well worthwhile to test for mutations in *PMS2*.

Very usefully, data has recently been presented on the underlying causes associated with patterns of MMR IHC in colorectal and endometrial tumours with MSI found in the setting of families in genetics clinics.[13] (Table 2) This also shows that by no means are all mutations necessarily associated with loss or abnormality of the corresponding protein.[13,14] Hence, patterns of MMR IHC abnormality are a guide to, but not an absolute indicator of the underlying genetic defect.

Table 2. Underlying causes of microsatellite instability in colorectal and endometrial cancers in genetics clinic patients, by associated pattern of MMR IHC abnormality.

Comprehensive data on consistency of IHC abnormality associated with specific mutations is lacking, but in the authors' experience it is variable: some mutations are associated with abnormal expression in perhaps 50% of tumours in a family, whereas with others it is 100%. In addition, in 1-2% of tumours that are tested, unusual combinations of abnormality may be seen, such as loss of MSH2 and PMS2, or three proteins, for reasons which are unclear (although some of the MMR genes contain coding microsatellites which may bias them to somatic mutations in cells with deficient MMR). Thus, pathologists should be aware that MMR IHC, like all tests, has finite sensitivity and specificity and some complex cases provide challenges in interpretation.

2.1.6 *Tumour tests in LS: Systematic testing of incident cases*

Ascertainment of LS by means of family history has a number of limitations, and suffers from considerable insensitivity and lack of specificity. Given that LS cancers are a cause of avoidable early onset death there is a very strong case for systematic testing of incident cancers to find the condition, the health economics of which has now been firmly established.[15] Because of this, and the prognostic and predictive information that detection of abnormal MMR can confer, the Royal College of Pathologists Minimum Dataset (2014) for Colorectal Cancer now states that

testing of all CRC up to age 50 for deficient MMR is required (for accredited services in the UK this is thus now effectively mandatory), and while the case is similarly made for testing all CRC between 50 and 70 y, testing is optional in this age range until commissioning is established.[16] Systematic testing of all CRC has been carried out for two years in Denmark, regardless of age, showing that ~3.3% of all CRC are due to LS. However, health economic analyses from both The Netherlands and the UK indicate that testing of CRC over 70y would not be cost-effective.

2.1.7 *Constitutional Mismatch Repair Disorder (CMMR-D)*

Individuals who inherit mutations in both copies of an MMR gene develop a condition known as Constitutional Mismatch Repair Disorder (CMMR-D). In its most severe form this is manifest as a propensity to brain cancer (mostly high-grade gliomas, but also includes supratentorial primitive neuroectodermal tumours and medulloblastomas), leukaemia and lymphoma with death by the age of 12 y.[17] The development of colorectal adenomas, sometimes amounting to polyposis, is also seen, as are cutaneous café-au-lait spots and other signs hitherto associated with NF1, such as freckling, Lisch nodules and neurofibromas. It is now appreciated that the first described cases, in consanguineous families, were this severe because the underlying mutations were in *MSH2* and *MLH1*. As cases due to *MSH6* and *PMS2* have been ascertained it is evident that CRC, small bowel and other LS-associated cancers develop in teenage, with individuals surviving into their third decade or beyond.

All cells in individuals with CMMR-D, tumours included, show loss of the respective MMR protein. This is diagnostic and another reason why all four MMR proteins should be assessed.[18]

CMMR-D shows recessive inheritance in the peculiar circumstance of the mutation carriers themselves exhibiting LS, a dominant condition, in analogous fashion to individuals with one *BRCA2* mutation having familial breast-ovarian cancer, while those with two *BRCA2* mutations have Fanconi anaemia, complementation group D1. Turcot's syndrome, characterised by brain cancer, adenomatous polyps and recessive inheritance is undoubtedly largely explained by CMMR-D, but new *APC* mutations (as seen in FAP, q.v. 2.2.1) are also possible.

2.2 Polyposes

2.2.1 *Familial Adenomatous Polyposis (FAP)*

FAP is the archetypal cancer predisposition syndrome, of singular importance in that it was one of the first human Mendelian genetic conditions to be identified, and was instrumental both in the concept of cancer arising from premalignant lesions (the adenoma-carcinoma sequence) and the development by Dukes of one of the first systems for cancer staging. It is a multisystem disorder due to dominantly inherited mutations in the *APC* gene, with a prevalence of 1:8500. The mutations are invariably protein truncating, causing the loss of beta-catenin binding sites in the expressed APC protein, with consequent dysregulation of wnt-signalling resulting in cellular dysplasia.(Fig. 3) Up to 10% of cases may be due to *de novo* mutations, but some of these are undiagnosed MAP.(see 2.3.3). The established adenomas and carcinomas are otherwise unremarkable, and there are no specific tests for FAP that can be carried out on them. However, multiple monocryptal adenomas are considered a helpful diagnostic feature.

Fig. 3 Sections of mouse small intestine

Hundreds to thousands of colorectal adenomas develop in teenage, resulting in an almost 100% lifetime risk of colorectal cancer if prophylactic total colectomy is not performed, usually in late teenage. Untreated, the average age of bowel cancer is 42y, but in families with thousands of polyps it is 29y. Small bowel adenomas also arise, characteristically in the duodenum and are an especial clinical problem, because prophylactic surgery is not possible and duodenal cancer carries a poor prognosis: indeed it is now one of the leading causes of death in FAP. Adenomas also develop in small bowel used to construct pouches post-colectomy. Rectal polyps arising after total colectomy and ileorectal anastomosis are managed until the risk of rectal cancer in the 5th decade of life becomes significant and resection is performed.

Desmoid tumours are another major cause of mortality and morbidity: they are histologically benign but clinically malignant. They can and do occur sporadically, but in FAP are more common intra-abdominally and around the trunk. They are indolent, unresponsive to chemotherapy, encroach on vital structures like small bowel and ureters and surgery is problematic, indeed colectomy may actually provoke or stimulate precursor lesions in the mesentery.[19]

Benign gastric fundic gland polyps are commonly seen in the stomach with occasional adenomas. Hepatoblastoma is seen in children, and hepatobiliary cancers in adults. Brain (usually medulloblastoma) and papillary thyroid carcinomas also occur at low frequency (1-3%). Epidermoid (sebaceous) and odontoid cysts are common as are osteomas, adrenal adenomas and supernumerary teeth. The constellation of colonic polyposis and extracolonic features was described by Gardner in the 1950's, but close examination reveals most if not all FAP patients have these features, so it is an obsolete term.

2.2.2 *Attenuated FAP (AFAP)*

Some families with mutations in particular parts of the *APC* gene develop fewer than 100 colorectal adenomas, the threshold for classical FAP, and some individuals may not develop any polyps. The average age of cancer is 55y. Other FAP-related features may not be seen, but some families can have a very high risk of desmoids. Only about 10% of individuals with <100 adenomas have a mutation in *APC*. As with FAP, there are no specific tumour tests for AFAP.

2.2.3 MUTYH-*associated polyposis (MAP)*

In 2003, recessive inheritance of mutations in a DNA repair enzyme called mutY homologue (*MUTYH*) was shown to cause adenomatous polyposis. Like other classic autosomal recessive DNA repair disorders, individuals inherit *MUTYH* mutations from both parents, who themselves are not affected. The phenotype overlaps with FAP and AFAP: patients develop up to a few hundred adenomas, but may not develop any, although they may still develop cancer. About 25% of those with >9 adenomas and without a dominant family history have MAP, as do 5% of those with 3 to 100 adenomas otherwise unselected. Upper GI polyposis certainly occurs, with risk of cancer, and

the rate of extra-intestinal cancers is modestly increased, in particular ovary, bladder and skin, but others may be involved. There are no specific tumour tests for MAP, but genetic testing for MAP is simplified by only needing to test for common mutations in the first instance, and so is relatively quick and inexpensive.

The loss of oxidative DNA damage repair in MAP results in innumerable mutations all over the genome, mostly G>T changes. Occasionally this may manifest as microsatellite instability (MSI) and thus make it appear as if the tumour might be due to Lynch syndrome, but a multiplicity of adenomas will be the clue that something other than LS is the cause.(see also 2.2.4)

2.2.4 *Polymerase-associated Polyposis (PAP)*

This condition has only been recently described and is being elucidated. Families with LS-like family histories, e.g. colorectal and endometrial cancers, plus multiple colorectal adenomas (variable, but up to approximately 50) have been found to have constitutional mutations affecting the proof-reading domains of DNA polymerases (*POLD1* and *POLE*). As yet there are no specific tests for PAP such as IHC for the respective polymerase proteins. Hence, diagnosis involves testing for Lynch and MAP with negative findings, including generally MSS tumours, and then testing the *POLD1* and *POLE* genes. However, PAP-related tumours accumulate innumerable point mutations all over the genome, so just as in MAP their tumours may occasionally show MSI, suggesting LS. Hence, it is important to consider the overall pattern of findings in a family in conjunction with genetics colleagues.

2.2.5 *Serrated Polyposis Syndrome (SPS)*

In SPS (previously known as Hyperplastic Polyposis Syndrome) multiple hyperplastic or serrated lesions (mostly hyperplastic polyps, but also many sessile serrated lesions/polyps/adenomas with a few serrated adenomas) are found in the large bowel, and there is an increased risk of bowel cancer.(Fig. 4)

Fig. 4 Tumours seen in Serrated Polyposis Syndrome.

If a patient fulfils any of the WHO criteria a diagnosis of SPS is made [20]:-

A At least five serrated lesions proximal to the sigmoid colon, two of which are greater than 10 mm in diameter, or

B Any number of serrated lesions occurring proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis, or

C More than 20 serrated lesions of any size distributed throughout the colon.

SPS shows a strong familial effect: first-degree relatives have a 5-fold increased risk of CRC. It appears more common in those of European descent, but the genetics remain to be determined. Smoking is associated with greater numbers of hyperplastic, but especially serrated lesions. CRC of serrated origin may have MSI, and so young onset CRCs arising in the setting of serrated polyposis can masquerade as a 'Lynch cancer' until the polyp phenotype is appreciated or a *BRAF* V600E mutation is found.[21]

2.2.6 *Peutz-Jeghers Polyposis (PJS)*

Peutz-Jeghers syndrome is due to inherited mutations in *STK11 (LKB1)*. The histology of PJS polyps is characteristic and diagnostic, consisting of branched or frond-like patterns of the muscularis mucosae smooth muscle, termed arborization.(Fig. 5) Cystic gland dilatation is observed, which may extend into the sub-mucosa and muscularis propria. Juvenile polyps, in contrast, have a lamina propria mostly lacking smooth muscle, but nonetheless the two types are often confused. Small bowel JPS polyps can exhibit 'pseudoinvasion' (epithelial misplacement) which can be mistaken for invasive carcinoma. Histological review should therefore be considered.

Fig. 5 Peutz-Jeghers polyp

PJS polyps typically affect the small bowel, leading to intussucception and obstruction. Few, if any, patients will get into their third decade without at least one laparotomy. It is therefore important that the condition be recognised and treated at expert centres which can offer the appropriate specialist care such as resecting as many polyps as possible at one operation. PJS patients are at risk of a variety of malignancies, especially of the GI tract, including pancreas, breast, uterine, and gonadal tumours with a risk of any cancer by age of 70 y of around 90%. The typical perioral freckling is also found around other centre line orifices but usually fades after the age of 20y.

In a single individual, a clinical diagnosis of PJS may be made when any one of the following is present [22]:

1. Two or more histologically confirmed PJ polyps.

2. Any number of PJ polyps detected in one individual who has a family history of PJS in close relative(s).

3. Characteristic mucocutaneous pigmentation in an individual who has a family history of PJS in close relative(s).

4. Any number of PJ polyps in an individual who also has characteristic mucocutaneous pigmentation.

2.2.7 *Juvenile Polyposis Syndrome (JPS)*

Juvenile polyps occur sporadically in childhood. They are delicate structures that are prone to haemorrhage, prolapse, and auto-amputation, so they not uncommonly cause anaemia and being fragile they are typically inflamed and thus are frequently misdiagnosed as inflammatory polyps.(Fig. 6) They occur throughout the colorectum, albeit with a slight preponderance in the

rectosigmoid colon. However, there is a group of individuals who develop multiple juvenile polyps, often with a family history of the same or bowel cancer – this is familial juvenile polyposis syndrome (JPS), due to either *SMAD4* or *BMPR1A* mutations. JPS is an autosomal dominant (AD) condition with variable penetrance, associated with an increased risk of colorectal cancer (CRC 10–38%) and of gastric and duodenal cancer (15–21%). Adenomatous change can be found in juvenile polyps and this may account for their malignant potential.[23]

Fig. 6 Juvenile polyp

In the absence of features suggesting Cowden syndrome, JPS is diagnosed when there are:-

• >5 juvenile polyps in the colon or rectum, or

• Juvenile polyps in other parts of the GI tract, or

• Any number of juvenile polyps and a positive family history.

2.2.8 *Hereditary Mixed Polyposis Syndrome* (HMPS)

This is a rare condition characterized by the development of a *mixture* of colorectal polyps in the same individual, including adenomas and serrated lesions, juvenile and JPS-like polyps, and *mixed* polyps with combinations of these features, e.g. adenomas with serrated features, hyperplastic polyps with adenomatous change, and juvenile polyps with hyperplastic or dysplastic features.(Fig. 7) It is due to abnormal expression of very large amounts of Gremlin, a protein involved in APC/wnt regulation in crypts in the bowel mucosa. A mutation outside of the *GREM1* gene, but close to it, causes upregulated ectopic expression. Thus, HMPS has a unique mechanism – the affected protein is not in itself mutant, rather its abnormal pattern of expression leads to dysplasia and malignancy. The original family affected with HMPS is of Ashkenazi Jewish origin, but mutations affecting Gremlin expression may well have occurred in other families, and there is evidence that common variants near *GREM1* influence CRC risk in the general population.[24]

Fig. 7 Examples of polyps seen in HMPS

2.2.9 *Cowden's Syndrome (CS)*

CS is due to constitutional *PTEN* mutations and the overarching term 'PTEN Hamartoma Tumour Syndrome' is proposed, because phenotypically overlapping conditions, such as Bannayan-Riley-Ruvalcaba, PTEN-related Proteus and Proteus-like syndromes are also caused by *PTEN* mutations, indeed often the exact same mutations as cause CS.[25] Adenomas, various hamartomas and other lesions, including lipomas, fibromas and characteristic ganglioneuromas are described in the large bowel in CS patients.(Fig. 8) The lifetime risk of CRC in CS appears to be modestly increased at about 9%.

Fig. 8 Cowden ganglioneuroma

Practice points

- A multidisciplinary team approach should be taken to the diagnosis of hereditary GI cancer, and Lynch syndrome in particular: a familial pattern in tumour and genetic findings may be evident when individual findings are less obviously significant.
- Give at least an approximate numerical estimate of polyp numbers, e.g. “<10” or “20-50”: use of the word ‘multiple’ without quantification is ambiguous and to be discouraged.
- If assessing DNA MMR by immunohistochemistry participate in EQA and test all four markers (MSH2, MLH1, MSH6 and PMS2).
- Abnormality of DNA MMR may be manifest as abnormal rather than simple loss of expression: e.g. patchy/weak or cytoplasmic and/or loss of nuclear staining.
- Patterns of MMR IHC abnormality are a guide to, but not an absolute indicator of the underlying genetic defect.
- Beware! Both MSI and MMR IHC testing are less sensitive when used on benign tumours, cancers at sites other than the colon, and when mutations in the *MSH6* and *PMS2* genes are the underlying cause. One may be abnormal when the other is not.
- Juvenile polyps are often misdiagnosed as “inflammatory”; juvenile and Peutz-Jeghers polyps are commonly confused.
- Young onset colorectal cancers arising in serrated polyps or serrated polyposis syndrome may masquerade as LS, because of the loss of MMR: take note of associated polyps.

Conflict of interest statement

IMF is an assessor for MMR IHC with the UK National External Quality Assurance Service for Immunocytochemistry and In Situ Hybridisation (UK NEQAS ICC; http://www.uknegasicc.ucl.ac.uk/), and Honorary Medical Advisor to *Lynch Syndrome UK* (http://www.lynch-syndrome-uk.org/). MJA is the founder and Lead assessor for MMR IHC with UK NEQAS ICC.

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