Human Molecular Genetics, 2021, Vol. 30, No. 20 R206–R224

https://doi.org/10.1093/hmg/ddab208
Advance Access Publication Date: 30 July 2021
Invited Review Article

INVITED REVIEW ARTICLE

From *APC* to the genetics of hereditary and familial colon cancer syndromes

Alisa P. Olkinuora[1,2,*], Päivi T. Peltomäki[1,2], Lauri A. Aaltonen[1,2,3] and Kristiina Rajamäki[1,3]

[1]Department of Medical and Clinical Genetics, Medicum, University of Helsinki, 00014 Helsinki, Finland, [2]iCAN Digital Precision Cancer Medicine Flagship, University of Helsinki, 00014 Helsinki, Finland and [3]Applied Tumor Genomics Research Program, Research Programs Unit, University of Helsinki, 00014 Helsinki, Finland

*To whom correspondence should be addressed. Tel: +358503129178; Fax: +3580294125105; Email: alisa.olkinuora@helsinki.fi

Abstract

Hereditary colorectal cancer (CRC) syndromes attributable to high penetrance mutations represent 9–26% of young-onset CRC cases. The clinical significance of many of these mutations is understood well enough to be used in diagnostics and as an aid in patient care. However, despite the advances made in the field, a significant proportion of familial and early-onset cases remains molecularly uncharacterized and extensive work is still needed to fully understand the genetic nature of CRC susceptibility. With the emergence of next-generation sequencing and associated methods, several predisposition loci have been unraveled, but validation is incomplete. Individuals with cancer-predisposing mutations are currently enrolled in life-long surveillance, but with the development of new treatments, such as cancer vaccinations, this might change in the not so distant future for at least some individuals. For individuals without a known cause for their disease susceptibility, prevention and therapy options are less precise. Herein, we review the progress achieved in the last three decades with a focus on how CRC predisposition genes were discovered. Furthermore, we discuss the clinical implications of these discoveries and anticipate what to expect in the next decade.

Introduction

Familial clustering of colorectal cancer (CRC) and gastrointestinal polyposis has been recognized for well over a century (1,2), but little was known about the heritable changes in this heterogeneous group of diseases until the last three decades. Familial clustering is observed in up to 25% of CRC cases (3) and twin studies have yielded heritability estimates of ∼35% (4).

Inherited predisposition to CRC because of high penetrance germline mutations (Table 1) has been implicated in 9–26% of patients diagnosed before the age of 50 years (5–9). Hereditary CRC syndromes caused by these mutations are phenotypically divided into polyposis and non-polyposis syndromes (Fig. 1). The more common non-polyposis syndromes include Lynch syndrome (OMIM#120435; LS), caused by DNA mismatch repair (MMR) gene mutations and resulting in a hypermutated tumor phenotype, and familial colorectal cancer type X (FCCTX) describing the remaining MMR-proficient cases with a heterogeneous genetic background. Polyposis syndromes are distinguished by adenomatous or non-adenomatous (hamartomatous/serrated) polyp histology (10). Of these, the causative gene for familial adenomatous polyposis (OMIM#175100; FAP), *APC*, was discovered first (11) (Fig. 2). CRC syndromes also involve predisposition to other tumors, including breast and gastrointestinal cancers in hamartomatous polyposes (12–14), endometrial cancer in LS (15,16), and fundic gland polyps (17) and desmoid tumors (18) in FAP.

Received: May 6, 2021. **Revised:** July 1, 2021. **Accepted:** July 5, 2021

© The Author(s) 2021. Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com.

Table 1. Established (in bold) and putative high penetrance genes associated with hereditary CRC and polyposis

Gene symbol	Gene name	Gene function	Cancer syndrome(s)	Mode of inheritance	Method of discovery	Type of genetic instability	PubMed ID	Ref. no.
APC	Adenomatous polyposis coli	Inhibitor of Wnt signaling pathway	FAP	Autosomal dominant	Chromosomal deletion	CIN	3 789 010 1 651 174 1 651 562 1 651 563 1 678 319	(21) (11) (26) (221) (222)
AXIN2	Axis inhibition protein 2	Inhibitor of Wnt signaling pathway	Familial tooth agenesis and attenuated familial polyposis	Autosomal dominant	Linkage and candidate gene analysis	CIN?	15 042 511	(62)
BMPR1A	BMP receptor, type IA	Activates SMAD transcrip-tional regula-tors	JPS FCCTX	Autosomal dominant	Linkage analysis	CIN (low)	11 381 269 (JPS) 21 640 116 (FCCTX)	(73) (JPS) (223) (FCCTX)
BRF1	BRF1 RNA polymerase III transcription initiation factor subunit	Activator of RNA poly-merase	FCCTX Cerebellofaciodental syndrome (CFDS)	Autosomal dominant	Exome sequencing	CIN	28 912 018 (FCCTX) 25 561 519 (CFDS)	(224) (FCCTX) (225) (CFDS)
EPCAM	Epithelial cell adhesion molecule	Cell adhesion	LS (*MSH2* hypermethylation from transcriptional read-through of *EPCAM*) Congenital tufting enteropathy (CTE)	Autosomal dominant (LS) Autosomal recessive (CTE)	Tumor phenotype	MSI (LS)	16 951 683 (LS) 19 098 912 (LS) 18 572 020 (CTE)	(226) (LS) (227) (LS) (228) (CTE)
ERCC6	ERCC excision repair 6, chromatin remodeling factor	Transcription-coupled nucleotide excision repair	FCCTX Cockayne's syndrome (CKS)	Autosomal domi-nant/codominant with WRN? (FCCTX) Autosomal recessive (CKS)	Exome sequencing	CIN	26 344 056 (FCCTX) 1 339 317 (CKS) 9 443 879 (CKS)	(83) (FCCTX) (229) (CKS) (230) (CKS)
FAF1	Fas-associated factor 1	DNA replica-tion	FCCTX	Autosomal dominant	Exome sequencing	CIN	32 179 092	(231)
FAN1	FANCD2- and FANCI-associated nuclease 1	DNA cross-link repair	FCCTX Karyomegalic interstitial nephritis (KMIN)	Autosomal dominant (FCCTX) Autosomal recessive (KMIN)	Exome sequencing	CIN	26 052 075 (FCCTX) 22 772 369 (KMIN)	(232) (FCCTX) (233) (KMIN)

Continued

Table 1. Continued

Gene symbol	Gene name	Gene function	Cancer syndrome(s)	Mode of inheritance	Method of discovery	Type of genetic instability	PubMed ID	Ref. no.
GALNT12	Polypeptide *N*-acetylgalactosaminyl-transferase 12	Oligosaccharide biosynthesis	FCCTX	Autosomal dominant (possibly dominant negative)	Candidate gene approach	?	19 617 566	(234)
GREM1	Gremlin 1, DAN family BMP antagonist	BMP antagonist	Mixed polyposis syndrome	Autosomal dominant	Candidate gene approach	CIN (low?)	22 561 515	(235)
MBD4	Methyl-CpG binding domain 4, DNA glycosylase	BER	MBD4-associated neoplasia syndrome (MANS)	Autosomal recessive	Whole-genome sequencing	CpG > TpG	30 049 810	(236) (237)
MLH1	MutL homolog 1	MMR	LS (monoallelic) Constitutional MMR deficiency syndrome (CMMRD; biallelic)	Autosomal dominant (LS) Autosomal recessive (CMMRD)	Linkage analysis	MSI-H Hypermutated	7 903 889 8 128 251 8 145 827	(53) (51) (52)
MLH3	MutL homolog 3	MMR recombination	MLH3-associated adenomatous polyposis (biallelic) Low-penetrance CRC (monoallelic)	Autosomal recessive (polyposis)	Exome sequencing (polyposis) Candidate gene approach (CRC)	CIN (polyposis) MSI-low (CRC)	30 573 798 (polyposis) 11 586 295 (CRC)	(91) (polyposis) (238) CRC
MRE11	MRE11 homolog, DSB nuclease	Telomere maintenance	FCCTX Ataxia-telangiectasia-like disorder (ATLD)	Autosomal dominant? Autosomal recessive (ATLD)	Exome sequencing	CIN?	27 329 137 (FCCTX) 10 612 394 (ATLD)	(239) (FCCTX) (240) (ATLD)
MSH2	MutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*)	MMR	LS (monoallelic) CMMRD; biallelic	Autosomal dominant (LS) Autosomal recessive (CMMRD)	Linkage analysis and tumor phenotype	MSI-H Hypermutated	8 484 120 8 252 616 8 261 515 8 484 121	(46) (47) (45) (57)
MSH3	MutS homolog 3	MMR	MSH3-associated adenomatous polyposis (biallelic)	Autosomal recessive (polyposis)	Exome sequencing	MSI (EMAST)	27 476 653	(90)
MSH6	MutS homolog 6	MMR	LS (monoallelic) CMMRD; biallelic	Autosomal dominant (LS) Autosomal recessive (CMMRD)	Candidate gene approach	MSI-H	9 354 786	(81)
MUTYH	MutY DNA glycosylase	BER	MUTYH-associated adenomatous polyposis	Autosomal recessive	Tumor phenotype	G:C > T:A	11 818 965	(38)

Continued

Table 1. Continued

Gene symbol	Gene name	Gene function	Cancer syndrome(s)	Mode of inheritance	Method of discovery	Type of genetic instability	PubMed ID	Ref. no.
NTHL1	Nth-like DNA glycosylase 1	BER	NTHL1-associated adenomatous polyposis	Autosomal recessive	Exome sequencing	C:G > T:A Hypermutated	25 938 944	(89)
OGG1	8-Oxoguanine DNA glycosylase	BER	FCCTX	Autosomal dominant/codominant	Candidate gene approach	G:C > T:A?	21 195 604	(114)
PMS2	PMS2 postmeiotic segregation increased 2	MMR	LS (monoallelic) CMMRD; biallelic	Autosomal dominant (LS) Autosomal recessive (CMMRD)	Candidate gene approach	MSI	8 072 530	(241)
POLD1	DNA polymerase delta 1, catalytic subunit	DNA replication	PPAP	Autosomal dominant	Linkage analysis and genome sequencing	Ultrahypermutated Possible MSI	23 263 490	(87)
POLE	DNA polymerase epsilon, catalytic subunit	DNA replication	PPAP	Autosomal dominant	Linkage analysis and genome sequencing	Ultrahypermutated Possible MSI	23 263 490	(87)
POLE2	DNA polymerase epsilon 2, catalytic subunit	DNA repair and replication	FCCTX Adenomatous polyposis	Autosomal dominant? (27329137) Autosomal recessive? (25529843)	Exome sequencing	Hypermutant?	25 529 843 27 329 137	(242) (239)
POT1	Protection of telomeres 1	Telomere maintenance	FCCTX Familial melanoma (FM) Familial glioma (FG) Li-Fraumeni-like syndrome (LFL)	Autosomal dominant?	Exome sequencing	CIN?	27 329 137 (FCCTX) 24 686 849 (FM) 24 686 846 (FM) 25 482 530 (FG) 26 403 419 (LFL)	(239) (FCCTX) (243) (FM) (244) (FM) (245) (FG) (246) (LFL)
PTEN	Phosphatase and tensin homolog	Phospatase	CS PTEN hamartoma tumor syndrome	Autosomal dominant	Linkage and candidate gene approach	CIN (low?)	8 673 088 9 072 974 9 140 396	(66) (247) (67)
RNF43	Ring finger protein 43	DNA damage response	Serrated polyposis syndrome	Autosomal dominant	Exome sequencing	CIN	24 512 911	(248)
RPS20	Ribosomal protein 20	Ribosome biogenesis	FCCTX DBA	Autosomal dominant	Exome sequencing	CIN?	24 941 021 (FCCTX) 32 790 018 (DBA)	(88) (FCCTX) (98) (DBA)

Continued

Table 1. Continued

Gene symbol	Gene name	Gene function	Cancer syndrome(s)	Mode of inheritance	Method of discovery	Type of genetic instability	PubMed ID	Ref. no.
SEMA4A	Semaphorin 4A	Semaphorin	FCCTX Retinis pigmentosa (RP)	Autosomal dominant (FCCTX) Autosomal recessive (RP)	Linkage analysis and exome sequencing	CIN	25 307 848 (FCCTX) 16 199 541 (RP)	(249) (FCCTX) (250) (RP)
SMAD4	SMAD family member 4	Cytoplasmic mediators of TGF-beta signaling	Juvenile polyposis syndrome	Autosomal dominant	Candidate gene approach	CIN (low?)	9 582 123	(71)
STK11	Serine/threonine kinase 11	AMPK activity regulator	PJS	Autosomal dominant	Chromosomal deletion	CIN (low)	8 988 175 9 428 765	(68) (69)
WRN	WRN RecQ like helicase	DNA DSB repair	FCCTX Werner syndrome	Autosomal dominant/co-dominant with ERCC6 (FCCTX) Autosomal recessive (Werner)	Exome sequencing (FCCTX) Candidate gene approach (Werner)	CIN	26 344 056 (FCCTX) 8 602 509 (Werner)	(83) (FCCTX) (251) (Werner)

In this anniversary review, we give an overview on genetics of hereditary CRC syndromes with a focus on Mendelian conditions and developments in genetic analysis techniques over the past three decades.

Clues From Molecular Tumor Phenotype

Chromosomal abnormalities have been attributed to the genesis of cancer for over a century (19), and deletions of the long arm of chromosome 5 (5q) have been recognized as the most frequent chromosome anomaly in secondary leukemia and in some solid cancers for some 40 years (20). As 5q was already a prominent factor in cancer development and familial clustering of colon polyposis was acknowledged, a clinical report of a Gardner syndrome patient with a *de novo* interstitial deletion within the 5q arm (21) provided the 'missing link' connecting 5q to polyposis predisposition. Soon, several research groups confirmed frequent allelic loss in 5q in CRC (22) and linked the adenomatous polyposis phenotype to the locus (23–25), leading to the detailed characterization of the *APC* gene (26) and initiating the hunt for gene defects responsible for inherited CRC and polyposes. Today, germline *APC* mutations are recognized as the underlying cause of FAP (Table 1); of these, 10–25% arise *de novo*. Typical of disorders with high *de novo* mutation rates (27), somatic *APC* mutations are sometimes restricted to certain tissues or organs, reflecting the timing of the mutation occurrence in embryogenesis. In these cases multiple genotypes are present in the patient, collectively referred to as genetic mosaicism. Patients with somatic *APC* mosaicism generally manifest as sporadic cases, as a disease phenotype only occurs when a substantial number of cells with the mutation are present in the target tissue. The detection of somatic *APC* mosaicism with conventional methods often proves difficult; the mutations might not be observable in samples derived from peripheral blood (28–30). Somatic *APC* mutations are commonplace in colorectal tumors and key players initiating colonic tumorigenesis (31–33).

Sometimes the landscape of somatic mutations can provide direct clues to the underlying germline mutation. In a British polyposis family negative for *APC* germline mutations, 11 tumors revealed somatic inactivation of *APC* because of G:C → T:A transversions (34). Previous efforts had identified spontaneous G:C → T:A transversions in *OGG1* (35), *MUTYH* (36,37), and *MTH* mutant *Escherichia coli* and *Saccharomyces cerevisiae*, which gave researchers an incentive to test the aforementioned genes for mutations. This resulted in the discovery of two *MUTYH* missense mutations in the family, p.Tyr165Cys and p.Gly382Asp, segregating with the disease in an autosomal recessive mode. Nowadays both variants are recognized as common causes for *MUTYH*-associated polyposis (OMIM#608456) when biallelic (34,38,39). Later mutational signature analyses have attributed the G:C → T:A transversions in *MUTYH*-deficient tumors to the mutational signatures 18 (40) and 36 (41). Tumors deficient of the base-excision repair protein *NTHL1* also have a conserved mutational signature characterized by C:G → T:A transitions (mutational signature 30) (42,43). Similarly, small insertions and deletions at repetitive sequences were consistently observed in tumors belonging to the spectrum of hereditary non-polyposis CRC (44), simultaneously attributed to genes in charge of MMR (45–47). This tumor phenotype is widely in use for diagnostic purposes as we will later discuss.

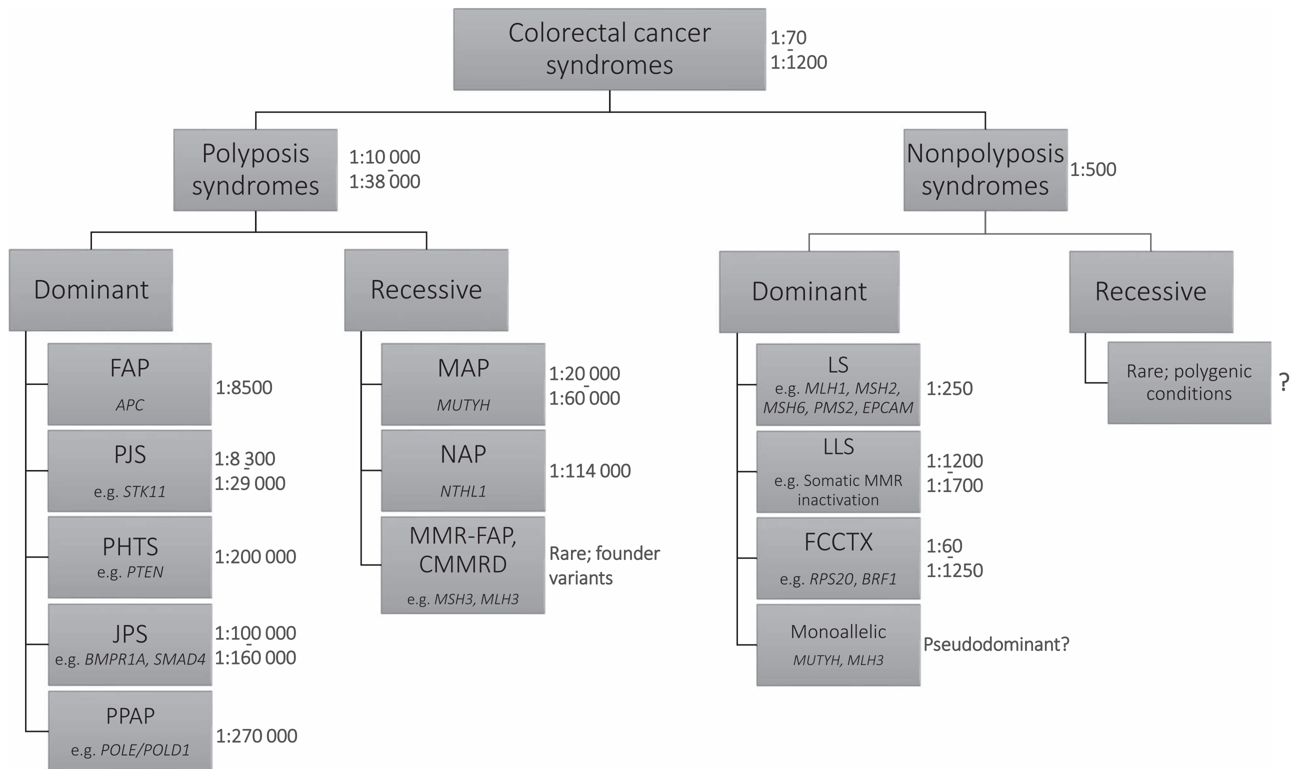

Figure 1. CRC syndromes. Division between syndromes is traditionally based on the number of and histopathology of intestinal polyps and mode of inheritance. The most common germline mutant genes for each syndrome are given. Ratios indicate estimates of prevalence of each syndrome based on literature (201–213).

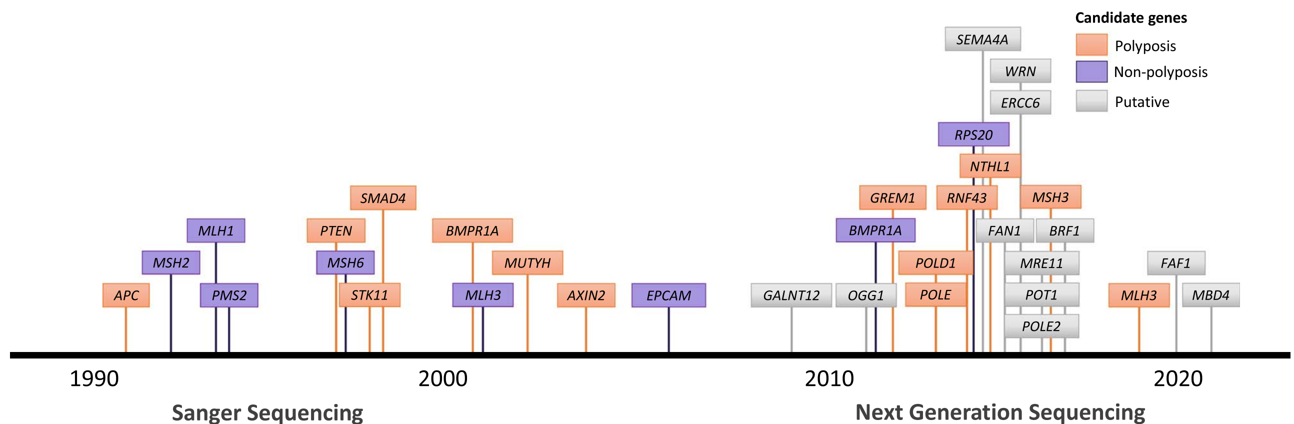

Figure 2. Timeline of CRC and polyposis susceptibility gene discoveries in the Sanger sequencing and NGS eras. Timeline assignment is based on the discovery of the gene as a CRC or polyposis susceptibility gene (the gene itself may have been identified before). Established susceptibility genes are colored and putative are indicated with a gray box.

Gene-Hunting With Linkage Analysis—Narrowing It Down

Genetic linkage analysis remains a powerful tool for rare variant detection when combined with whole-genome sequencing (48,49) and has played an important role in gene discoveries made in CRC syndromes. LS is caused by germline mutations in MMR genes *MLH1* (42% of unique variants), *MSH2* (33%), *MSH6* (18%), and *PMS2* (7.5%) (50) (Table 1, Fig. 3). *MSH2* and *MLH1* were the first LS genes discovered in 1993–1994 (45,47,51,52), fueled by mapping of the respective predisposing loci by genetic linkage analysis (46,53,54). For *MSH2*, two large LS kindreds were studied using 345 microsatellite markers across the genome, revealing highly significant linkage with marker *D2S123* at 2p15-16 (46). For *MLH1*, restriction fragment length polymorphisms and microsatellite markers studied in Swedish LS families revealed a predisposing locus at 3p21.3-23 (53,54). Subsequent gene identification by positional cloning was aided by the evolving understanding of MMR proteins in bacteria and yeast, providing a connection with the instability of DNA microsatellite markers observed in LS and other human tumors (55–61).

AXIN2 locus underlying oligodontia-CRC syndrome (OMIM#608615) (Table 1) was discovered in a genome-wide search utilizing microsatellite markers in an affected Finnish family (62). Linkage was excluded for *APC* and candidate loci associated with tooth development, pointing to a region in chromosome 17. *AXIN2* (17q24.1) became the strongest candidate owing to its role in WNT signaling (Fig. 3) and

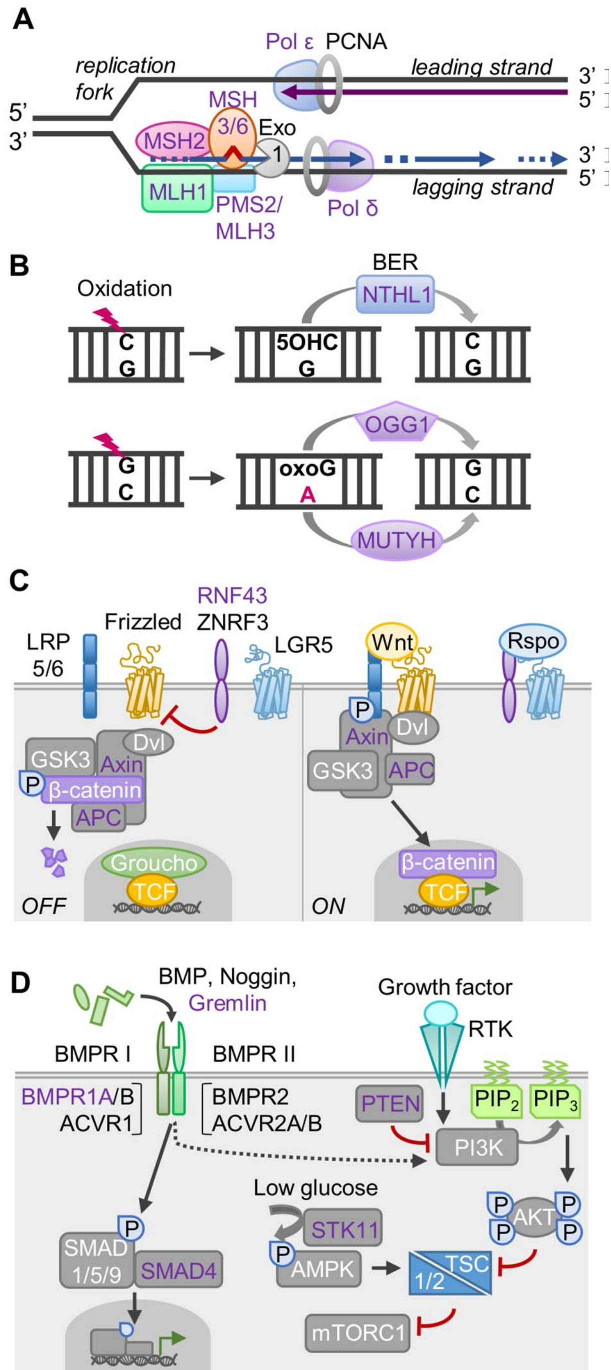

Figure 3. Key biological pathways associated with hereditary CRC syndromes. Genes with known germline mutations are shown in purple font. (**A**) Fidelity of DNA replication depends primarily on DNA polymerases ε and δ for correct base pairing and proofreading exonuclease activity. Any persisting base–base mispairs or insertion–deletion loops both on the leading and lagging strands are subsequently targeted by MMR proteins. MSH2:MSH3/6 dimers recognize the error. Interaction with MLH1:PMS2 triggers downstream repair events initiated by removal of the erroneous DNA by exonuclease 1 (Exo1). (**B**) Oxidation, alkylation, and deamination of DNA bases are repaired by base excision repair (BER) pathway. Eleven DNA glycosylases recognize and remove the damaged/mispaired bases; these are divided into monofunctional (e.g. MUTYH) and bifunctional (e.g. NTHL1, OGG1) glycosylases based on the presence of additional endonuclease activity. Their function is exemplified by repair of oxidative 8-oxoguanine (oxoG) and 5-hydroxycytosine (5-OHC) lesions. (**C**) Wnt signaling regulates cell

somatic mutations in CRC (63,64). Sequencing revealed an *AXIN2* nonsense mutation segregating with the phenotype (62) as later confirmed in unrelated cases (65).

The genes for the main hamartomatous polyposis syndromes—Cowden syndrome (OMIM#158350; CS), Peutz-Jeghers syndrome (OMIM#175200; PJS), and juvenile polyposis syndrome (OMIM#174900; JPS)—were all identified through linkage studies. For CS, a traditional linkage and candidate gene analysis identified *PTEN* as the culprit gene (66,67) (Table 1, Fig. 3). In case of PJS, a targeted linkage analysis based on allelic loss detected in PJS polyps by comparative genomic hybridization revealed the disease locus, followed by fine mapping and systematic sequencing of transcripts in the region to identify the gene, *LKB1/STK11* (68,69). For JPS, targeted linkage analysis excluded loci previously associated with CRC and closely related hamartomatous polyposis syndromes, revealing linkage to 18q21.1 (70). This led to the discovery of *SMAD4* (18q21.2) mutations (71), but these were found to explain only a minority of the cases (72). Genome-wide linkage analysis in four JPS families without *SMAD4* or *PTEN* mutations resulted in the discovery of germline *BMPR1A* (10q23.2) nonsense mutations in all families (73). Together, mutations in *SMAD4* and *BMPR1A*, both involved in transforming growth factor beta (TGF-β) signaling, account for 50–60% of JPS cases (74) (Table 1, Fig. 3).

Candidate Genes Pinpointed by Biological Function

Existing knowledge of biological functions of candidate genes can greatly facilitate predisposition gene discoveries. Human MSH6 was identified as a 160 kDa protein dimerizing with MSH2 in a mismatch-binding factor purified from HeLa cells (75–77) (Fig. 3). Both proteins were required for restoring MMR in extracts of hypermutable CRC cell lines (76–78). Sequence analysis of proteolytic peptides from p160 allowed cDNA identification, revealing homology to human and yeast MSH2 and *E. coli* MutS (75–77). An *Msh6*-deficient mouse model gave the first evidence for a role in cancer susceptibility (79). While initial attempts to find germline mutations of *MSH6* in 20 LS kindreds failed (78), mutations were soon found in atypical LS cases with weak family history (80,81). In contrast to *MLH1* or *MSH2* mutant tumors, microsatellite instability (MSI) is not always detected in *MSH6* mutant tumors (79,82), in agreement with its preferential role in the repair of single-nucleotide mismatches (76–78).

In a study of 25 patients with familial CRC of undefined genetic basis (83), mostly falling into the category of FCCTX (Fig. 1), the authors hypothesized to find mutations predisposing to DNA double-strand breaks (DSBs) based on earlier reports on replication stress as the underlying mechanism of chromosomal instability in CRC (84). Compared with controls, CRC patient T cells had increased susceptibility to DSBs under ultraviolet radiation or aphidicolin-induced replication stress, and exome sequencing showed significantly higher frequency of rare disruptive germline mutations in DSB repair-related genes, found in 25 genes in 17/20 patients (83). Heterozygous mutations in *WRN* (genome stability during DNA repair) and *ERCC6* (transcription-coupled nucleotide excision repair) found in one of the patients were functionally validated, further supporting a role for constitutional defects in suppression of DSBs as a new molecular class of hereditary CRC. Homozygous mutations cause Werner

(OMIM#277700) and Cockayne (OMIM#133540) syndromes, respectively, the former associated with various neoplasms and the latter with heightened sensitivity to ultraviolet. Interestingly, the *WRN*-encoded DNA helicase was suggested to have a cell/tissue type specific role in MMR (85), and a related RecQ family DNA helicase *BLM* directly interacts with MSH6 (86).

From Single Genes to the Exome and Genome

The emergence of new sequencing techniques has enabled scientists to make advances in oncogenomics, especially in the study of somatic changes in tumors. Nevertheless, success in research of CRC predisposition has been limited. In conjunction with family information, be it in the form of linkage or other methods, a handful of novel causative genes have been identified in the last decade (Fig. 2, Table 1) (87–91). One of the successful results is the characterization of polymerase proofreading-associated polyposis (PPAP) by discovery of the associated genes: *POLE* and *POLD1* (OMIM#615083 and OMIM#612591, respectively). Whole exome sequencing (WES) analysis of 15 mutation negative index cases with polyposis and family history of CRC, as well as some of their family members, initially left the researchers empty-handed: after excluding common and benign variants across multiple families, no susceptibility genes were identified (87). However, previous linkage data from a few families in this study (92,93) pinpointed the analysis to *POLE* and *POLD1* genes, resulting in the identification of three families wherein the mutations segregated with the disease. Subsequent research has further defined the roles of *POLE* and *POLD1* in familial CRC and polyposis (94).

The genetic background of FCCTX has been a particularly difficult one to crack: the underlying genes have eluded geneticists worldwide despite being under investigation for almost 20 years (95). In a similar fashion to the discovery of PPAP, a combination of linkage analysis and WES identified a truncating variant of *RPS20* in association with MMR-proficient CRC in a four-generation family (88,95). Subsequent studies have since identified three *RPS20* mutations in familial CRC cases (96,97) and two in Diamond-Blackfan anemia (DBA) patients (98). Available experience suggests that only a small fraction of FCCTX susceptibility genes have been discovered so far, and that private genes and mutations are common, strengthening the idea of the heterogeneous genetic background of FCCTX (94,99).

WES has become the main approach to detect novel cancer susceptibility genes and has enabled much of the recent success in the discovery of novel CRC and polyposis susceptibility genes. In addition to *POLE*, *POLD1*, and *RPS20*, recent successful reports of novel CRC and polyposis susceptibility variant discoveries include *NTHL1* (89), *MSH3* (90), and *MLH3* (91) (Table 1). While WES is able to detect (or exclude) mutations in exons of protein-coding genes, a disadvantage of the method is that only ∼2% of the genome is covered and many of the regulatory elements controlling the expression of these genes remain unrevealed. For example, intronic variants resulting in a splicing acceptor or donor domains can create pseudoexons, intronic sequences inserted in the mature mRNA of a gene. Detection of such variants is impossible with just WES and requires simultaneous transcriptome and whole genome-based approaches. Few cases of *APC* pseudoexons have been reported (100,101).

Toward Understanding 'the Missing Heritability'—Oligogenic and Polygenic Etiology

Though much has been learned from hereditary CRC, mutations in known susceptibility genes explain only a fraction of familial CRC risk, and multiple explanations for this 'missing heritability' have been proposed (102–105). Genome-wide association studies (GWAS) continue to uncover novel susceptibility loci and common low-risk variants associated with CRC at the population level, further shaping our view of the genetic landscape of CRC (105,106). A recent effort combining whole-genome sequencing and imputation into GWAS data almost doubled the number of known association signals for sporadic CRC into ∼100 independent signals at >80 CRC risk loci (107), implicating novel pathways and gene families in CRC risk. These GWAS findings may provide further clues into etiology of hereditary CRC syndromes, as genes previously linked with hereditary syndromes have been found to harbor also common CRC risk variants with weaker effects (105). Common variants may modify CRC risk in patients with hereditary CRC syndromes, possibly explaining some of the observed differences in the ages of cancer diagnosis and disease presentation among individuals carrying the same high-penetrance mutation (108–112). Moreover, combinations of multiple common low-penetrance variants may work synergistically to substantially contribute to overall disease heritability. A recent example is from serrated polyposis syndrome, a hereditary CRC predisposition with a poorly understood genetic basis, which was analyzed in a case–control study for contribution of 65 common low-penetrance CRC risk variants, reporting significant associations of the condition with seven of these (113). These findings, together with emerging reports on di- or oligogenic inheritance via rare moderately penetrant variants (114–117), highlight the complex genetic make-up of hereditary CRC.

Clinical Implications

Molecular diagnostics of hereditary CRC has advanced significantly in the past decades, enabling today efficient screening strategies to identify individuals at high risk for referral to genetic testing and specialized cancer surveillance programs for each hereditary CRC predisposition (118). The genetic testing

development and stemness and is commonly hyperactivated in cancer. In the absence of secreted Wnt ligands, a cytoplasmic destruction complex directs β-catenin for proteasomal degradation and RNF43/ZNRF3 ubiquitin ligases downregulate Frizzled receptors. Upon receptor binding of Wnts, β-catenin is released and translocates to the nucleus, where it binds TCF/LEF transcription factors and displaces the Groucho repressor to activate target gene transcription. Binding of R-spondins (Rspo) to LGR5 inhibits RNF43/ZNRF3, enhancing Wnt signaling. (**D**) TGF-β signaling restricts proliferation of colonic epithelial cells. Bone morphogenetic proteins (BMPs) are TGF-β superfamily ligands that trigger receptor dimerization and activation via trans-phosphorylation, resulting in SMAD-dependent target gene transcription. Also, non-SMAD signaling pathways, including the PI3K–AKT–mTOR pathway, can be activated. Growth factor signaling via receptor tyrosine kinases (RTK) activates PI3K to generate phosphoinositide-3,4,5-triphosphate (PIP_3), resulting in AKT-mediated derepression of mTOR complex 1 (mTORC1). mTOR integrates nutrient and growth factor signals, promoting cell growth upon activation. PTEN lipid phosphatase antagonizes this pathway by converting PIP_3 back to PIP_2. Conversely, low glucose conditions trigger the STK11-dependent activation of AMPK, suppressing mTORC1. See references (214–220) for further details.

of affected individuals and predictive testing of their at-risk relatives, combined with intensive cancer surveillance, has an enormous cancer-preventive potential in these families, while also reducing unnecessary procedures and anxiety in family members who are found mutation negative.

Compared with polyposis syndromes, LS lacks distinctive endoscopic features and presents a particular challenge for diagnostics. Diagnosis was initially based on so-called Amsterdam criteria formulated in 1991 (119,120); these focused on family history and age of onset and were highly useful for selection of families for scientific gene identification studies. More practical and less stringent Bethesda guidelines (121,122) followed, utilizing tumor MSI for the selection of patients for further genetic testing. Both criteria became suboptimal for identifying LS cases as tools for genetic diagnostics advanced, prompting recommendations to offer genetic testing to all individuals with newly diagnosed CRC (123,124). The discovery of a high frequency of DNA replication errors in LS tumors compared with normal tissue paved the way for universal screening based on MSI (57,125–127). MSI testing involves polymerase chain reaction (PCR) amplification of a set of short repetitive DNA sequences (microsatellites) to determine changes in their length in tumors (128). Apart from LS, MSI occurs in ∼12% of sporadic CRCs because of defective MMR caused by *MLH1* promoter methylation or, rarely, bi-allelic somatic mutations of MMR genes (129,130). The former can be excluded by testing the tumors for the *BRAF*-V600E mutation and/or *MLH1* promoter hypermethylation (131). Immunohistochemistry for detecting loss of expression of MMR proteins in tumor tissue is another prevalent LS screening approach (132–134). MSI testing and immunohistochemistry identify LS cases with an estimated clinical sensitivity of 85/83% and specificity of 90/89%, respectively (124). Subsequent detection of germline mutation in an MMR gene by sequencing then establishes the diagnosis of LS.

In recent years, the sequencing of each established hereditary CRC gene individually has become increasingly replaced by next-generation sequencing (NGS)-based diagnostic gene panels. These allow simultaneous germline mutation testing of a wide selection of CRC predisposition genes in a cost-effective manner in suspected CRC/polyposis syndrome cases, supporting utility as a first line test (135). The overlapping clinical presentation and genetic heterogeneity in CRC syndromes may also be better addressed by panel testing. The diagnostic yield and clinical actionability of results from CRC panel testing vary depending on the cohort selected for testing, genes included in the panel, and variant interpretation (6–8,136–147). In cohorts of unselected CRC patients representing different populations and ethnicities, panel testing of 25, 18, 73, 83, and 27 cancer-predisposing genes identified pathogenic or likely pathogenic germline mutations in 9.9%, 2%, 18.1%, 15.5%, and 3.3% of the patients, respectively (136,137,143,144,147). A large variety of commercial CRC gene panels are available (148). These show inconsistent criteria for inclusion of genes, including both high and low penetrance genes with highly variable levels of supporting evidence (148). To address these and other concerns, the Collaborative Group of the Americas on Inherited Gastrointestinal Cancer recently published a position statement proposing a minimal set of 11 genes to be included on a multigene panel for evaluation of hereditary CRC/polyposis; *MLH1, MSH2, MSH6, PMS2, EPCAM, APC, BMPR1A, MUTYH, PTEN, STK11,* and *SMAD4* (149). The statement also recommends an additional set of 16 genes to be considered for panel testing (149), including genes with low-to-moderate CRC risk, preliminary but limited data on CRC risk, or actionable mutations without proven causation in CRC. Notably, this set includes several genes with germline mutations not traditionally associated with CRC/polyposis, including *TP53, CHEK2,* and *BRCA1/2*, highlighting the utility of a broader analysis of hereditary cancer genes in diagnostic panels.

The hypermutable state in MSI tumors causes accumulation of frameshift mutations that generate neoantigens, immunogenic peptides recognized as non-self by the immune system (150–156). Neoantigen burden is significantly increased in MSI versus microsatellite stable (MSS) CRCs and in LS versus sporadic MSI CRCs (154,155). The neoantigens can be presented at the tumor cell surface in human leukocyte antigen type I (HLA I) molecules, eliciting antitumor cytotoxic T cell responses (150–152,154,157,158). These observations likely explain the high level of lymphocyte infiltration long known as a hallmark of MSI CRCs (159,160) and more recently shown to be a predictor of better prognosis (154,161) similar to MSI (162). The highly immunogenic tumor microenvironment in MSI CRCs is balanced by elevated expression of several immune checkpoint molecules that block antitumor T cell responses, making this subgroup an excellent candidate for treatment using immune checkpoint inhibitors (163,164). Clinical studies have consistently demonstrated efficacy of immune checkpoint inhibition with anti-PD-1 antibodies (pembrolizumab, nivolumab) in MSI tumors from colorectum and many other tissues (165–171). Both drugs have received US Food and Drug Administration approval for treatment of patients with MSI-high or MMR-deficient metastatic CRC, followed by approval of nivolumab combination immunotherapy with anti-CTLA-4 antibody (ipilimumab) for the same indication (169). Similar to MSI, also the presence of *POLE* and *POLD1* mutations, associated with an ultramutator phenotype in tumors, may predict survival benefit from immune checkpoint therapy in CRC and other cancers (172,173).

Conclusions and Future Perspectives

Advances in the dissection of hereditary CRC syndromes offer a remarkable example of how the understanding of human disease has improved in the past 30 years. Interestingly, with the new knowledge, the borders between established CRC syndromes have become blurrier: for example, evidence shows that some LS patients can mimic attenuated FAP (91,174) and few families exhibiting digenic inheritance of CRC have been reported (114,117). Thus, genotype–phenotype diversity in inherited CRC will likely continue to be a source of difficulty for diagnostics (140) and treatment (175). Much further work will be required to better understand the key components of CRC susceptibility and optimal management strategies for most syndromes, though effective targeted treatments already exist for some of the affected signaling pathways (Fig. 3). For example, patients with *APC* germline mutations respond well to treatment with the nonsteroidal anti-inflammatory drug sulindac and COX inhibitor celecoxib. In these patients, adenoma burden was significantly reduced (176), likely because of the suppression of canonical WNT/β-catenin signaling (177,178). Very recent breakthroughs have been made in LS vaccination by exploiting the recurrent frameshift-induced neoantigens consequent of the defective MMR machinery (179). Dense lymphocyte infiltration accompanies the MSI tumors of LS patients (159,160,180) and specific T cell reactivity to frameshift peptides is detectable already in healthy LS family members without a history of cancer (158). These observations, combined with the fact that LS carcinomas often lose HLA I expression likely because of

a selection pressure (181,182), suggest that the LS patient's immune system effectively recognizes MMR-deficient cells and may control tumor outgrowth. Therefore, vaccination is an attractive option for cancer prevention in the future. Moreover, vaccination could direct the development of MSI tumors toward a surgically curable phenotype by increasing the immunoselective pressure and likelihood of HLA I loss via mutations in beta-2-microglobulin (182); these mutations have been associated with an excellent prognosis with a low risk of relapse (183) and metastases (184,185). The first clinical trials for frameshift peptide vaccines are promising, with low toxicity and high immune response (179).

Similar to the use of MSI-induced frameshift products in LS-diagnosis, tumor-specific mutation spectra or signatures have laid the groundwork for connections between the molecular profiles of cancers and the tumor development (186). One notable advance in the last decade is the detailed characterization of mutational signatures across different cancer types (187,188). As each signature is a reflection of the mutational processes in cancer, especially prominent in samples whose DNA repair machinery is defective (35–37,40,41,43,189), they can serve as a hint toward the underlying cancer-causing mechanisms and serve as potential diagnostic or prognostic biomarkers (190). Furthermore, mutational signatures may provide evidence for accurate classification of variants of unknown significance as pathogenic or benign (191). For example, owing to the discovery of the mutational signature 30 in a breast cancer sample, a discovery of a germline mutation of *NTHL1* was made (42). Finding characteristic features, such as MSI or specific mutational signatures, in tumor samples could reflect the germline defect in molecularly unexplained familial CRC or polyposis cases, acting as a starting point for finding the underlying cause of disease. Unfortunately, availability of tumor DNA is often a limiting factor for NGS-based studies. Liquid biopsies could be a reliable source of tumor DNA for such purposes, as significant numbers of clinically actionable variants have been present in cell-free DNA samples when tumor samples were either unavailable or had not yielded any results (192). Similar conclusions can be drawn from studies utilizing mutation-specific PCR (193) and methylation analysis (194,195) of cell-free DNA from patients with metastatic disease. MSI status and mutational signatures observed in cell-free DNA seem to reflect those of the primary tumor as well (196,197).

Despite major advances in the field, CRC remains one of the leading causes of cancer-related death and a major economic burden (198,199) that personalized medicine is thought to alleviate (200). The decreasing costs of genomic research technologies bring the onset of precision medicine closer to reality as more research is carried out. Our current knowledge of germline and somatic changes in CRC predisposition has given us meaningful clinical predictors for the best-characterized syndromes; however, extending this to the rarer or lesser-known syndromes and understanding the genotype–phenotype correlations present in recognized syndromes is still awaiting further research. This includes the analysis of the noncoding genome and disease-modifying variants, which are anticipated to shed light onto the susceptibility to cancer as well as its progression and treatment. The work of cancer genomics projects, such as The Cancer Genome Atlas, The International Cancer Genome Consortium, or the Pan-Cancer Analysis of Whole Genomes Consortium, will likely deepen our understanding of CRC through the development of research tools and techniques able to identify generalized patterns and characteristics of cancer, such as mutational signatures, contributing to the missing factors prohibiting detailed molecular profiling of patient-matched cancer and interpretation of variants of unknown significance.

Currently, many of the syndromes are not surgically curable, and patients need life-long monitoring. With the rate of new technological advances, the next decade will be exciting and will likely yield a better understanding of the genetic and environmental determinants of CRC susceptibility, the phenotype–genotype correlations of the already established susceptibility genes, and optimal management of heritable CRC in its various forms.

Conflicts of Interest statement. None declared.

Funding

The Biomedicum Helsinki Foundation (to A.P.O.); The Finnish Center of Excellence in Tumor Genetics (to L.A.A.); the Jane and Aatos Erkko Foundation (to P.T.P.); the Academy of Finland (grant number 294643 to P.T.P.); the Academy of Finland's professorship (L.A.A.); the Cancer Foundation Finland (to P.T.P. and L.A.A.); iCAN Digital Precision Cancer Medicine Flagship (to L.A.A.); the Sigrid Juselius Foundation (to P.T.P. and L.A.A.); The Doctoral Programme of Biomedicine of Helsinki University offered a paid doctoral student position to A.P.O.

References

1. Bülow, S., Berk, T. and Neale, K. (2006) The history of familial adenomatous polyposis. *Fam. Cancer*, **5**, 213–220.
2. Boland, C.R. and Lynch, H.T. (2013) The history of Lynch syndrome. *Fam. Cancer*, **12**, 145–157.
3. Kerber, R.A., Neklason, D.W., Samowitz, W.S. and Burt, R.W. (2005) Frequency of familial colon cancer and hereditary nonpolyposis colorectal cancer (Lynch syndrome) in a large population database. *Fam. Cancer*, **4**, 239–244.
4. Lichtenstein, P., Holm, N.V., Verkasalo, P.K., Iliadou, A., Kaprio, J., Koskenvuo, M., Pukkala, E., Skytthe, A. and Hemminki, K. (2000) Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. *N. Engl. J. Med.*, **343**, 78–85.
5. Mork, M.E., Nancy You, Y., Ying, J., Bannon, S.A., Lynch, P.M., Rodriguez-Bigas, M.A. and Vilar, E. (2015) High prevalence of hereditary cancer syndromes in adolescents and young adults with colorectal cancer. *J. Clin. Oncol.*, **33**, 3544–3549.
6. DeRycke, M.S., Gunawardena, S., Balcom, J.R., Pickart, A.M., Waltman, L.A., French, A.J., McDonnell, S., Riska, S.M., Fogarty, Z.C., Larson, M.C. *et al.* (2017) Targeted sequencing of 36 known or putative colorectal cancer susceptibility genes. *Mol. Genet. Genomic Med.*, **5**, 553–569.
7. Pearlman, R., Frankel, W.L., Swanson, B., Zhao, W., Yilmaz, A., Miller, K., Bacher, J., Bigley, C., Nelsen, L., Goodfellow, P.J. *et al.* (2017) Prevalence and spectrum of germline cancer susceptibility gene mutations among patients with early-onset colorectal cancer. *JAMA Oncol.*, **3**, 464–471.
8. Stoffel, E.M., Koeppe, E., Everett, J., Ulintz, P., Kiel, M., Osborne, J., Williams, L., Hanson, K., Gruber, S.B. and Rozek, L.S. (2018) Germline genetic features of young individuals with colorectal cancer. *Gastroenterology*, **154**, 897–905.e1.
9. Daca Alvarez, M., Quintana, I., Terradas, M., Mur, P., Balaguer, F. and Valle, L. (2021) The inherited and familial component of early-onset colorectal cancer. *Cell*, **10**.
10. Geboes, K., De Hertogh, G., Van Caillie, M.-A. and Van Eyken, P. (2007) Non-adenomatous colorectal polyposis syndromes. *Curr. Diag. Pathol.*, **13**, 479–489.

11. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L. and Robertson, M. (1991) Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, **66**, 589–600.
12. van Lier, M.G.F., Wagner, A., Mathus-Vliegen, E.M.H., Kuipers, E.J., Steyerberg, E.W. and van Leerdam, M.E. (2010) High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. *Am. J. Gastroenterol.*, **105**, 1258–1264 author reply 1265.
13. Howe, J.R., Mitros, F.A. and Summers, R.W. (1998) The risk of gastrointestinal carcinoma in familial juvenile polyposis. *Ann. Surg. Oncol.*, **5**, 751–756.
14. Pilarski, R. (2019) Hamartoma tumor syndrome: a clinical overview. *Cancer*, **11**, 844.
15. Møller, P., Seppälä, T., Bernstein, I., Holinski-Feder, E., Sala, P., Evans, D.G., Lindblom, A., Macrae, F., Blanco, I., Sijmons, R. *et al.* (2017) Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. *Gut*, **66**, 464–472.
16. Møller, P., Seppälä, T.T., Bernstein, I., Holinski-Feder, E., Sala, P., Gareth Evans, D., Lindblom, A., Macrae, F., Blanco, I., Sijmons, R.H. *et al.* (2018) Cancer risk and survival in carriers by gene and gender up to 75 years of age: a report from the prospective Lynch syndrome database. *Gut*, **67**, 1306–1316.
17. Abraham, S.C., Nobukawa, B., Giardiello, F.M., Hamilton, S.R. and Wu, T.T. (2000) Fundic gland polyps in familial adenomatous polyposis: neoplasms with frequent somatic adenomatous polyposis coli gene alterations. *Am. J. Pathol.*, **157**, 747–754.
18. Righetti, A.E.M., Jacomini, C., Parra, R.S., de Almeida, A.L.N.R., Rocha, J.J.R. and Féres, O. (2011) Familial adenomatous polyposis and desmoid tumors. *Clinics*, **66**, 1839–1842.
19. Boveri, T. (1914) Zur Frage der Entstehung maligner Tumoren. *Naturwissenschaften*, **2**, 676–679.
20. Van den Berghe, H., Vermaelen, K., Mecucci, C., Barbieri, D. and Tricot, G. (1985) The 5q-anomaly. *Cancer Genet. Cytogenet.*, **17**, 189–255.
21. Herrera, L., Kakati, S., Gibas, L., Pietrzak, E. and Sandberg, A.A. (1986) Gardner syndrome in a man with an interstitial deletion of 5q. *Am. J. Med. Genet.*, **25**, 473–476.
22. Solomon, E., Voss, R., Hall, V., Bodmer, W.F., Jass, J.R., Jeffreys, A.J., Lucibello, F.C., Patel, I. and Rider, S.H. (1987) Chromosome 5 allele loss in human colorectal carcinomas. *Nature*, **328**, 616–619.
23. Leppert, M., Dobbs, M., Scambler, P., O'Connell, P., Nakamura, Y., Stauffer, D., Woodward, S., Burt, R., Hughes, J. and Gardner, E. (1987) The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science*, **238**, 1411–1413.
24. Bodmer, W.F., Bailey, C.J., Bodmer, J., Bussey, H.J.R., Ellis, A., Gorman, P., Lucibello, F.C., Murday, V.A., Rider, S.H., Scambler, P. *et al.* (1987) Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature*, **328**, 614–616.
25. Leppert, M., Burt, R., Hughes, J.P., Samowitz, W., Nakamura, Y., Woodward, S., Gardner, E., Lalouel, J.M. and White, R. (1990) Genetic analysis of an inherited predisposition to colon cancer in a family with a variable number of adenomatous polyps. *N. Engl. J. Med.*, **322**, 904–908.
26. Kinzler, K.W., Nilbert, M.C., Su, L.K., Vogelstein, B., Bryan, T.M., Levy, D.B., Smith, K.J., Preisinger, A.C., Hedge, P. and McKechnie, D. (1991) Identification of FAP locus genes from chromosome 5q21. *Science*, **253**, 661–665.
27. Hall, J.G. (1988) Review and hypotheses: somatic mosaicism: observations related to clinical genetics. *Am. J. Hum. Genet.*, **43**, 355–363.
28. Aretz, S., Stienen, D., Friedrichs, N., Stemmler, S., Uhlhaas, S., Rahner, N., Propping, P. and Friedl, W. (2007) Somatic APC mosaicism: a frequent cause of familial adenomatous polyposis (FAP). *Hum. Mutat.*, **28**, 985–992.
29. Spier, I., Drichel, D., Kerick, M., Kirfel, J., Horpaopan, S., Laner, A., Holzapfel, S., Peters, S., Adam, R., Zhao, B. *et al.* (2016) Low-level APC mutational mosaicism is the underlying cause in a substantial fraction of unexplained colorectal adenomatous polyposis cases. *J. Med. Genet.*, **53**, 172–179.
30. Hes, F.J., Nielsen, M., Bik, E.C., Konvalinka, D., Wijnen, J.T., Bakker, E., Vasen, H.F.A., Breuning, M.H. and Tops, C.M.J. (2008) Somatic APC mosaicism: an underestimated cause of polyposis coli. *Gut*, **57**, 71–76.
31. Rustin, R.B., Jagelman, D.G., McGannon, E., Fazio, V.W., Lavery, I.C. and Weakley, F.L. (1990) Spontaneous mutation in familial adenomatous polyposis. *Dis Colon Rectum*, **33**, 52–55.
32. Ripa, R., Bisgaard, M.L., Bülow, S. and Nielsen, F.C. (2002) De novo mutations in familial adenomatous polyposis (FAP). *Eur. J. Hum. Genet.*, **10**, 631–637.
33. Aretz, S., Uhlhaas, S., Caspari, R., Mangold, E., Pagenstecher, C., Propping, P. and Friedl, W. (2004) Frequency and parental origin of de novo APC mutations in familial adenomatous polyposis. *Eur. J. Hum. Genet.*, **12**, 52–58.
34. Cleary, S.P., Cotterchio, M., Jenkins, M.A., Kim, H., Bristow, R., Green, R., Haile, R., Hopper, J.L., LeMarchand, L., Lindor, N. *et al.* (2009) Germline MutY human homologue mutations and colorectal cancer: a multisite case-control study. *Gastroenterology*, **136**, 1251–1260.
35. Thomas, D., Scot, A.D., Barbey, R., Padula, M. and Boiteux, S. (1997) Inactivation of OGG1 increases the incidence of G:C–>T:A transversions in *Saccharomyces cerevisiae*: evidence for endogenous oxidative damage to DNA in eukaryotic cells. *Mol. Gen. Genet.*, **254**, 171–178.
36. Nghiem, Y., Cabrera, M., Cupples, C.G. and Miller, J.H. (1988) The mutY gene: a mutator locus in *Escherichia coli* that generates G.C—-T.A transversions. *Proc. Natl. Acad. Sci. U. S. A.*, **85**, 2709–2713.
37. Moriya, M. and Grollman, A.P. (1993) Mutations in the mutY gene of *Escherichia coli* enhance the frequency of targeted G:C–>T:A transversions induced by a single 8-oxoguanine residue in single-stranded DNA. *Mol. Gen. Genet.*, **239**, 72–76.
38. Al-Tassan, N., Chmiel, N.H., Maynard, J., Fleming, N., Livingston, A.L., Williams, G.T., Hodges, A.K., Davies, D.R., David, S.S., Sampson, J.R. *et al.* (2002) Inherited variants of MYH associated with somatic G:C–>T:A mutations in colorectal tumors. *Nat. Genet.*, **30**, 227–232.
39. Nielsen, M., Joerink-van de Beld, M.C., Jones, N., Vogt, S., Tops, C.M., Vasen, H.F.A., Sampson, J.R., Aretz, S. and Hes, F.J. (2009) Analysis of MUTYH genotypes and colorectal phenotypes in patients with MUTYH-associated polyposis. *Gastroenterology*, **136**, 471–476.
40. Pilati, C., Shinde, J., Alexandrov, L.B., Assié, G., André, T., Hélias-Rodzewicz, Z., Ducoudray, R., Le Corre, D., Zucman-Rossi, J., Emile, J.-F. *et al.* (2017) Mutational signature analysis identifies MUTYH deficiency in colorectal cancers and adrenocortical carcinomas. *J. Pathol.*, **242**, 10–15.
41. Viel, A., Bruselles, A., Meccia, E., Fornasarig, M., Quaia, M., Canzonieri, V., Policicchio, E., Urso, E.D., Agostini, M., Genuardi, M. *et al.* (2017) A specific mutational signature

associated with DNA 8-oxoguanine persistence in MUTYH-defective colorectal cancer. *EBioMedicine*, **20**, 39–49.
42. Drost, J., van Boxtel, R., Blokzijl, F., Mizutani, T., Sasaki, N., Sasselli, V., de Ligt, J., Behjati, S., Grolleman, J.E., van Wezel, T. *et al.* (2017) Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. *Science*, **358**, 234–238.
43. Grolleman, J.E., de Voer, R.M., Elsayed, F.A., Nielsen, M., Weren, R.D.A., Palles, C., Ligtenberg, M.J.L., Vos, J.R., Ten Broeke, S.W., de Miranda, N.F.C.C. *et al.* (2019) Mutational signature analysis reveals NTHL1 deficiency to cause a multi-tumor phenotype. *Cancer Cell*, **35**, 256–266.e5.
44. Peltomäki, P., Lothe, R.A., Aaltonen, L.A., Pylkkänen, L., Nyström-Lahti, M., Seruca, R., David, L., Holm, R., Ryberg, D. and Haugen, A. (1993) Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res.*, **53**, 5853–5855.
45. Leach, F.S., Nicolaides, N.C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomäki, P., Sistonen, P., Aaltonen, L.A. and Nyström-Lahti, M. (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell*, **75**, 1215–1225.
46. Peltomäki, P., Aaltonen, L.A., Sistonen, P., Pylkkänen, L., Mecklin, J.P., Järvinen, H., Green, J.S., Jass, J.R., Weber, J.L. and Leach, F.S. (1993) Genetic mapping of a locus predisposing to human colorectal cancer. *Science*, **260**, 810–812.
47. Fishel, R., Lescoe, M.K., Rao, M.R., Copeland, N.G., Jenkins, N.A., Garber, J., Kane, M. and Kolodner, R. (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell*, **75**, 1027–1038.
48. Ott, J., Wang, J. and Leal, S.M. (2015) Genetic linkage analysis in the age of whole-genome sequencing. *Nat. Rev. Genet.*, **16**, 275–284.
49. Teare, M.D., Dawn Teare, M. and Barrett, J.H. (2005) Genetic linkage studies. *Lancet*, **366**, 1036–1044.
50. Plazzer, J.P., Sijmons, R.H., Woods, M.O., Peltomäki, P., Thompson, B., Den Dunnen, J.T. and Macrae, F. (2013) The InSiGHT database: utilizing 100 years of insights into Lynch syndrome. *Fam. Cancer*, **12**, 175–180.
51. Papadopoulos, N., Nicolaides, N.C., Wei, Y.F., Ruben, S.M., Carter, K.C., Rosen, C.A., Haseltine, W.A., Fleischmann, R.D., Fraser, C.M. and Adams, M.D. (1994) Mutation of a mutL homolog in hereditary colon cancer. *Science*, **263**, 1625–1629.
52. Bronner, C.E., Baker, S.M., Morrison, P.T., Warren, G., Smith, L.G., Lescoe, M.K., Kane, M., Earabino, C., Lipford, J. and Lindblom, A. (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature*, **368**, 258–261.
53. Lindblom, A., Tannergård, P., Werelius, B. and Nordenskjöld, M. (1993) Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. *Nat. Genet.*, **5**, 279–282.
54. Tannergård, P., Zabarovsky, E., Stanbridge, E., Nordenskjöld, M. and Lindblom, A. (1994) Sublocalization of a locus at 3p21.3-23 predisposing to hereditary nonpolyposis colon cancer. *Hum. Genet.*, **94**, 210–214.
55. Levinson, G. and Gutman, G.A. (1987) High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. *Nucleic Acids Res.*, **15**, 5323–5338.
56. Strand, M., Prolla, T.A., Liskay, R.M. and Petes, T.D. (1993) Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature*, **365**, 274–276.
57. Aaltonen, L.A., Peltomäki, P., Leach, F.S., Sistonen, P., Pylkkänen, L., Mecklin, J.P., Järvinen, H., Powell, S.M., Jen, J. and Hamilton, S.R. (1993) Clues to the pathogenesis of familial colorectal cancer. *Science*, **260**, 812–816.
58. Ionov, Y., Peinado, M.A., Malkhosyan, S., Shibata, D. and Perucho, M. (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature*, **363**, 558–561.
59. Thibodeau, S.N., Bren, G. and Schaid, D. (1993) Microsatellite instability in cancer of the proximal colon. *Science*, **260**, 816–819.
60. Risinger, J.I., Berchuck, A., Kohler, M.F., Watson, P., Lynch, H.T. and Boyd, J. (1993) Genetic instability of microsatellites in endometrial carcinoma. *Cancer Res.*, **53**, 5100–5103.
61. Han, H.J., Yanagisawa, A., Kato, Y., Park, J.G. and Nakamura, Y. (1993) Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res.*, **53**, 5087–5089.
62. Lammi, L., Arte, S., Somer, M., Jarvinen, H., Lahermo, P., Thesleff, I., Pirinen, S. and Nieminen, P. (2004) Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am. J. Hum. Genet.*, **74**, 1043–1050.
63. Behrens, J., Jerchow, B.A., Würtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kühl, M., Wedlich, D. and Birchmeier, W. (1998) Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. *Science*, **280**, 596–599.
64. Liu, W., Dong, X., Mai, M., Seelan, R.S., Taniguchi, K., Krishnadath, K.K., Halling, K.C., Cunningham, J.M., Boardman, L.A., Qian, C. *et al.* (2000) Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating beta-catenin/TCF signalling. *Nat. Genet.*, **26**, 146–147.
65. Marvin, M.L., Mazzoni, S.M., Herron, C.M., Edwards, S., Gruber, S.B. and Petty, E.M. (2011) AXIN2-associated autosomal dominant ectodermal dysplasia and neoplastic syndrome. *Am. J. Med. Genet. A*, **155A**, 898–902.
66. Nelen, M.R., Padberg, G.W., Peeters, E.A., Lin, A.Y., van den Helm, B., Frants, R.R., Coulon, V., Goldstein, A.M., van Reen, M.M., Easton, D.F. *et al.* (1996) Localization of the gene for Cowden disease to chromosome 10q22-23. *Nat. Genet.*, **13**, 114–116.
67. Liaw, D., Marsh, D.J., Li, J., Dahia, P.L., Wang, S.I., Zheng, Z., Bose, S., Call, K.M., Tsou, H.C., Peacocke, M. *et al.* (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat. Genet.*, **16**, 64–67.
68. Hemminki, A., Tomlinson, I., Markie, D., Järvinen, H., Sistonen, P., Björkqvist, A.M., Knuutila, S., Salovaara, R., Bodmer, W., Shibata, D. *et al.* (1997) Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. *Nat. Genet.*, **15**, 87–90.
69. Hemminki, A., Markie, D., Tomlinson, I., Avizienyte, E., Roth, S., Loukola, A., Bignell, G., Warren, W., Aminoff, M., Höglund, P. *et al.* (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*, **391**, 184–187.
70. Howe, J.R., Ringold, J.C., Summers, R.W., Mitros, F.A., Nishimura, D.Y. and Stone, E.M. (1998) A gene for familial juvenile polyposis maps to chromosome 18q21.1. *Am. J. Hum. Genet.*, **62**, 1129–1136.
71. Howe, J.R., Roth, S., Ringold, J.C., Summers, R.W., Järvinen, H.J., Sistonen, P., Tomlinson, I.P., Houlston, R.S., Bevan, S.,

Mitros, F.A. *et al.* (1998) Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science*, **280**, 1086–1088.

72. Houlston, R., Bevan, S., Williams, A., Young, J., Dunlop, M., Rozen, P., Eng, C., Markie, D., Woodford-Richens, K., Rodriguez-Bigas, M.A. *et al.* (1998) Mutations in DPC4 (SMAD4) cause juvenile polyposis syndrome, but only account for a minority of cases. *Hum. Mol. Genet.*, **7**, 1907–1912.
73. Howe, J.R., Bair, J.L., Sayed, M.G., Anderson, M.E., Mitros, F.A., Petersen, G.M., Velculescu, V.E., Traverso, G. and Vogelstein, B. (2001) Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. *Nat. Genet.*, **28**, 184–187.
74. Brosens, L.A., Langeveld, D., van Hattem, W.A., Giardiello, F.M. and Offerhaus, G.J.A. (2011) Juvenile polyposis syndrome. *World J. Gastroenterol.*, **17**, 4839–4844.
75. Hughes, M.J. and Jiricny, J. (1992) The purification of a human mismatch-binding protein and identification of its associated ATPase and helicase activities. *J. Biol. Chem.*, **267**, 23876–23882.
76. Palombo, F., Gallinari, P., Iaccarino, I., Lettieri, T., Hughes, M., D'Arrigo, A., Truong, O., Hsuan, J.J. and Jiricny, J. (1995) GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. *Science*, **268**, 1912–1914.
77. Drummond, J.T., Li, G.M., Longley, M.J. and Modrich, P. (1995) Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. *Science*, **268**, 1909–1912.
78. Papadopoulos, N., Nicolaides, N.C., Liu, B., Parsons, R., Lengauer, C., Palombo, F., D'Arrigo, A., Markowitz, S., Willson, J.K. and Kinzler, K.W. (1995) Mutations of GTBP in genetically unstable cells. *Science*, **268**, 1915–1917.
79. Edelmann, W., Yang, K., Umar, A., Heyer, J., Lau, K., Fan, K., Liedtke, W., Cohen, P.E., Kane, M.F., Lipford, J.R. *et al.* (1997) Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. *Cell*, **91**, 467–477.
80. Akiyama, Y., Sato, H., Yamada, T., Nagasaki, H., Tsuchiya, A., Abe, R. and Yuasa, Y. (1997) Germ-line mutation of the hMSH6/GTBP gene in an atypical hereditary nonpolyposis colorectal cancer kindred. *Cancer Res.*, **57**, 3920–3923.
81. Miyaki, M., Konishi, M., Tanaka, K., Kikuchi-Yanoshita, R., Muraoka, M., Yasuno, M., Igari, T., Koike, M., Chiba, M. and Mori, T. (1997) Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. *Nat. Genet.*, **17**, 271–272.
82. Wu, Y., Berends, M.J., Mensink, R.G., Kempinga, C., Sijmons, R.H., van Der Zee, A.G., Hollema, H., Kleibeuker, J.H., Buys, C.H. and Hofstra, R.M. (1999) Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. *Am. J. Hum. Genet.*, **65**, 1291–1298.
83. Arora, S., Yan, H., Cho, I., Fan, H.-Y., Luo, B., Gai, X., Bodian, D.L., Vockley, J.G., Zhou, Y., Handorf, E.A. *et al.* (2015) Genetic variants that predispose to DNA double-strand breaks in lymphocytes from a subset of patients with familial colorectal carcinomas. *Gastroenterology*, **149**, 1872–1883.e9.
84. Burrell, R.A., McClelland, S.E., Endesfelder, D., Groth, P., Weller, M.-C., Shaikh, N., Domingo, E., Kanu, N., Dewhurst, S.M., Gronroos, E. *et al.* (2013) Replication stress links structural and numerical cancer chromosomal instability. *Nature*, **494**, 492–496.
85. Bennett, S.E., Umar, A., Oshima, J., Monnat, R.J., Jr. and Kunkel, T.A. (1997) Mismatch repair in extracts of Werner syndrome cell lines. *Cancer Res.*, **57**, 2956–2960.
86. Pedrazzi, G., Bachrati, C.Z., Selak, N., Studer, I., Petkovic, M., Hickson, I.D., Jiricny, J. and Stagljar, I. (2003) The Bloom's syndrome helicase interacts directly with the human DNA mismatch repair protein hMSH6. *Biol. Chem.*, **384**, 1155–1164.
87. Palles, C., Cazier, J.-B., Howarth, K.M., Domingo, E., Jones, A.M., Broderick, P., Kemp, Z., Spain, S.L., Guarino, E., Salguero, I. *et al.* (2013) Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat. Genet.*, **45**, 136–144.
88. Nieminen, T.T., O'Donohue, M.-F., Wu, Y., Lohi, H., Scherer, S.W., Paterson, A.D., Ellonen, P., Abdel-Rahman, W.M., Valo, S., Mecklin, J.-P. *et al.* (2014) Germline mutation of RPS20, encoding a ribosomal protein, causes predisposition to hereditary nonpolyposis colorectal carcinoma without DNA mismatch repair deficiency. *Gastroenterology*, **147**, 595–598.e5.
89. Weren, R.D.A., Ligtenberg, M.J.L., Kets, C.M., de Voer, R.M., Verwiel, E.T.P., Spruijt, L., van Zelst-Stams, W.A.G., Jongmans, M.C., Gilissen, C., Hehir-Kwa, J.Y. *et al.* (2015) A germline homozygous mutation in the base-excision repair gene NTHL1 causes adenomatous polyposis and colorectal cancer. *Nat. Genet.*, **47**, 668–671.
90. Adam, R., Spier, I., Zhao, B., Kloth, M., Marquez, J., Hinrichsen, I., Kirfel, J., Tafazzoli, A., Horpaopan, S., Uhlhaas, S. *et al.* (2016) Exome sequencing identifies biallelic MSH3 germline mutations as a recessive subtype of colorectal adenomatous polyposis. *Am. J. Hum. Genet.*, **99**, 337–351.
91. Olkinuora, A., Nieminen, T.T., Mårtensson, E., Rohlin, A., Ristimäki, A., Koskenvuo, L., Lepistö, A., Swedish Extended Genetic Analysis of Colorectal Neoplasia (SWEN) Study Group, Gebre-Medhin, S., Nordling, M. *et al.* (2019) Biallelic germline nonsense variant of MLH3 underlies polyposis predisposition. *Genet. Med.*, **21**, 1868–1873.
92. Kemp, Z., Carvajal-Carmona, L., Spain, S., Barclay, E., Gorman, M., Martin, L., Jaeger, E., Brooks, N., Bishop, D.T., Thomas, H. *et al.* (2006) Evidence for a colorectal cancer susceptibility locus on chromosome 3q21-q24 from a high-density SNP genome-wide linkage scan. *Hum. Mol. Genet.*, **15**, 2903–2910.
93. Papaemmanuil, E., Carvajal-Carmona, L., Sellick, G.S., Kemp, Z., Webb, E., Spain, S., Sullivan, K., Barclay, E., Lubbe, S., Jaeger, E. *et al.* (2008) Deciphering the genetics of hereditary non-syndromic colorectal cancer. *Eur. J. Hum. Genet.*, **16**, 1477–1486.
94. Valle, L., de Voer, R.M., Goldberg, Y., Sjursen, W., Försti, A., Ruiz-Ponte, C., Caldés, T., Garré, P., Olsen, M.F., Nordling, M. *et al.* (2019) Update on genetic predisposition to colorectal cancer and polyposis. *Mol. Asp. Med.*, **69**, 10–26.
95. Lindor, N.M., Rabe, K., Petersen, G.M., Haile, R., Casey, G., Baron, J., Gallinger, S., Bapat, B., Aronson, M., Hopper, J. *et al.* (2005) Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. *JAMA*, **293**, 1979–1985.
96. Broderick, P., Dobbins, S.E., Chubb, D., Kinnersley, B., Dunlop, M.G., Tomlinson, I. and Houlston, R.S. (2017) Validation of recently proposed colorectal cancer susceptibility gene variants in an analysis of families and patients-a systematic review. *Gastroenterology*, **152**, 75–77.e4.
97. Thompson, B.A., Snow, A.K., Koptiuch, C., Kohlmann, W.K., Mooney, R., Johnson, S., Huff, C.D., Yu, Y., Teerlink, C.C., Feng, B.-J. *et al.* (2020) A novel ribosomal protein S20 variant in a

family with unexplained colorectal cancer and polyposis. *Clin. Genet.*, **97**, 943–944.
98. Bhar, S., Zhou, F., Reineke, L.C., Morris, D.K., Khincha, P.P., Giri, N., Mirabello, L., Bergstrom, K., Lemon, L.D., Williams, C.L. *et al.* (2020) Expansion of germline RPS20 mutation phenotype to include Diamond-Blackfan anemia. *Hum. Mutat.*, **41**, 1918–1930.
99. Peltomäki, P., Olkinuora, A. and Nieminen, T.T. (2020) Updates in the field of hereditary nonpolyposis colorectal cancer. *Expert Rev. Gastroenterol. Hepatol.*, **14**, 707–720.
100. Nieminen, T.T., Pavicic, W., Porkka, N., Kankainen, M., Järvinen, H.J., Lepistö, A. and Peltomäki, P. (2016) Pseudoexons provide a mechanism for allele-specific expression of APC in familial adenomatous polyposis. *Oncotarget*, **7**, 70685–70698.
101. Spier, I., Horpaopan, S., Vogt, S., Uhlhaas, S., Morak, M., Stienen, D., Draaken, M., Ludwig, M., Holinski-Feder, E., Nöthen, M.M. *et al.* (2012) Deep intronic APC mutations explain a substantial proportion of patients with familial or early-onset adenomatous polyposis. *Hum. Mutat.*, **33**, 1045–1050.
102. Aaltonen, L., Johns, L., Järvinen, H., Mecklin, J.-P. and Houlston, R. (2007) Explaining the familial colorectal cancer risk associated with mismatch repair (MMR)-deficient and MMR-stable tumors. *Clin. Cancer Res.*, **13**, 356–361.
103. Win, A.K., Jenkins, M.A., Dowty, J.G., Antoniou, A.C., Lee, A., Giles, G.G., Buchanan, D.D., Clendenning, M., Rosty, C., Ahnen, D.J. *et al.* (2017) Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol. Biomark. Prev.*, **26**, 404–412.
104. Schubert, S.A., Morreau, H., de Miranda, N.F.C.C. and van Wezel, T. (2020) The missing heritability of familial colorectal cancer. *Mutagenesis*, **35**, 221–231.
105. Peters, U., Bien, S. and Zubair, N. (2015) Genetic architecture of colorectal cancer. *Gut*, **64**, 1623–1636.
106. Tenesa, A. and Dunlop, M.G. (2009) New insights into the aetiology of colorectal cancer from genome-wide association studies. *Nat. Rev. Genet.*, **10**, 353–358.
107. Huyghe, J.R., Bien, S.A., Harrison, T.A., Kang, H.M., Chen, S., Schmit, S.L., Conti, D.V., Qu, C., Jeon, J., Edlund, C.K. *et al.* (2019) Discovery of common and rare genetic risk variants for colorectal cancer. *Nat. Genet.*, **51**, 76–87.
108. Talseth-Palmer, B.A., Wijnen, J.T., Grice, D.M. and Scott, R.J. (2013) Genetic modifiers of cancer risk in Lynch syndrome: a review. *Fam. Cancer*, **12**, 207–216.
109. Donald, N., Malik, S., McGuire, J.L. and Monahan, K.J. (2018) The association of low penetrance genetic risk modifiers with colorectal cancer in lynch syndrome patients: a systematic review and meta-analysis. *Fam. Cancer*, **17**, 43–52.
110. Fahed, A.C., Wang, M., Homburger, J.R., Patel, A.P., Bick, A.G., Neben, C.L., Lai, C., Brockman, D., Philippakis, A., Ellinor, P.T. *et al.* (2020) Polygenic background modifies penetrance of monogenic variants for tier 1 genomic conditions. *Nat. Commun.*, **11**, 3635.
111. Wiik, M.U., Evans, T.-J., Belhadj, S., Bolton, K.A., Dymerska, D., Jagmohan-Changur, S., Capellá, G., Kurzawski, G., Wijnen, J.T., Valle, L. *et al.* (2021) A genetic variant in telomerase reverse transcriptase (TERT) modifies cancer risk in Lynch syndrome patients harbouring pathogenic MSH2 variants. *Sci. Rep.*, **11**, 11401.
112. Jenkins, M.A., Buchanan, D.D., Lai, J., Makalic, E., Dite, G.S., Win, A.K., Clendenning, M., Winship, I.M., Hayes, R.B., Huyghe, J.R. *et al.* (2021) Assessment of a polygenic risk score for colorectal cancer to predict risk of Lynch syndrome colorectal cancer. *JNCI Cancer Spectr*, **5**, kab022.
113. Arnau-Collell, C., Soares de Lima, Y., Díaz-Gay, M., Muñoz, J., Carballal, S., Bonjoch, L., Moreira, L., Lozano, J.J., Ocaña, T., Cuatrecasas, M. *et al.* (2020) Colorectal cancer genetic variants are also associated with serrated polyposis syndrome susceptibility. *J. Med. Genet.*, **57**, 677–682.
114. Morak, M., Massdorf, T., Sykora, H., Kerscher, M. and Holinski-Feder, E. (2011) First evidence for digenic inheritance in hereditary colorectal cancer by mutations in the base excision repair genes. *Eur. J. Cancer*, **47**, 1046–1055.
115. Ciavarella, M., Miccoli, S., Prossomariti, A., Pippucci, T., Bonora, E., Buscherini, F., Palombo, F., Zuntini, R., Balbi, T., Ceccarelli, C. *et al.* (2018) Somatic APC mosaicism and oligogenic inheritance in genetically unsolved colorectal adenomatous polyposis patients. *Eur. J. Hum. Genet.*, **26**, 387–395.
116. Fernández-Rozadilla, C., Álvarez-Barona, M., Quintana, I., López-Novo, A., Amigo, J., Cameselle-Teijeiro, J.M., Roman, E., Gonzalez, D., Llor, X., Bujanda, L. *et al.* (2021) Exome sequencing of early-onset patients supports genetic heterogeneity in colorectal cancer. *Sci. Rep.*, **11**, 11135.
117. Schubert, S.A., Ruano, D., Tiersma, Y., Drost, M., de Wind, N., Nielsen, M., van Hest, L.P., Morreau, H., de Miranda, N.F.C.C. and van Wezel, T. (2020) Digenic inheritance of MSH6 and MUTYH variants in familial colorectal cancer. *Genes Chromosomes Cancer*, **59**, 697–701.
118. Stoffel, E.M. and Richard Boland, C. (2015) Genetics and genetic testing in hereditary colorectal cancer. *Gastroenterology*, **149**, 1191–1203.e2.
119. Vasen, H.F., Mecklin, J.P., Khan, P.M. and Lynch, H.T. (1991) The International Collaborative group on hereditary nonpolyposis colorectal cancer (ICG-HNPCC). *Dis Colon Rectum*, **34**, 424–425.
120. Vasen, H.F., Watson, P., Mecklin, J.P. and Lynch, H.T. (1999) New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology*, **116**, 1453–1456.
121. Rodriguez-Bigas, M.A., Boland, C.R., Hamilton, S.R., Henson, D.E., Jass, J.R., Khan, P.M., Lynch, H., Perucho, M., Smyrk, T., Sobin, L. *et al.* (1997) A National Cancer Institute workshop on hereditary nonpolyposis colorectal cancer syndrome: meeting highlights and Bethesda guidelines. *J. Natl. Cancer Inst.*, **89**, 1758–1762.
122. Umar, A., Boland, C.R., Terdiman, J.P., Syngal, S., de la Chapelle, A., Rüschoff, J., Fishel, R., Lindor, N.M., Burgart, L.J., Hamelin, R. *et al.* (2004) Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J. Natl. Cancer Inst.*, **96**, 261–268.
123. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (2009) Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet. Med.*, **11**, 35–41.
124. Palomaki, G.E., McClain, M.R., Melillo, S., Hampel, H.L. and Thibodeau, S.N. (2009) EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genet. Med.*, **11**, 42–65.
125. Aaltonen, L.A., Peltomäki, P., Mecklin, J.P., Järvinen, H., Jass, J.R., Green, J.S., Lynch, H.T., Watson, P., Tallqvist, G. and

Juhola, M. (1994) Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res.*, **54**, 1645–1648.
126. Aaltonen, L.A., Salovaara, R., Kristo, P., Canzian, F., Hemminki, A., Peltomäki, P., Chadwick, R.B., Kääriäinen, H., Eskelinen, M., Järvinen, H. *et al.* (1998) Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N. Engl. J. Med.*, **338**, 1481–1487.
127. Salovaara, R., Loukola, A., Kristo, P., Kääriäinen, H., Ahtola, H., Eskelinen, M., Härkönen, N., Julkunen, R., Kangas, E., Ojala, S. *et al.* (2000) Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J. Clin. Oncol.*, **18**, 2193–2200.
128. Zhang, L. (2008) Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing. *J. Mol. Diagn.*, **10**, 301–307.
129. Boland, C.R., Richard Boland, C. and Goel, A. (2010) Microsatellite instability in colorectal cancer. *Gastroenterology*, **138**, 2073–2087.e3.
130. Carethers, J.M. (2014) Differentiating Lynch-like from Lynch syndrome. *Gastroenterology*, **146**, 602–604.
131. Parsons, M.T., Buchanan, D.D., Thompson, B., Young, J.P. and Spurdle, A.B. (2012) Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. *J. Med. Genet.*, **49**, 151–157.
132. Hampel, H., Frankel, W.L., Martin, E., Arnold, M., Khanduja, K., Kuebler, P., Nakagawa, H., Sotamaa, K., Prior, T.W., Westman, J. *et al.* (2005) Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N. Engl. J. Med.*, **352**, 1851–1860.
133. Piñol, V., Castells, A., Andreu, M., Castellví-Bel, S., Alenda, C., Llor, X., Xicola, R.M., Rodríguez-Moranta, F., Payá, A., Jover, R. *et al.* (2005) Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. *JAMA*, **293**, 1986–1994.
134. Shia, J. (2008) Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. the utility of immunohistochemistry. *J. Mol. Diagn.*, **10**, 293–300.
135. Gallego, C.J., Shirts, B.H., Bennette, C.S., Guzauskas, G., Amendola, L.M., Horike-Pyne, M., Hisama, F.M., Pritchard, C.C., Grady, W.M., Burke, W. *et al.* (2015) Next-generation sequencing panels for the diagnosis of colorectal cancer and polyposis syndromes: a cost-effectiveness analysis. *J. Clin. Oncol.*, **33**, 2084–2091.
136. Yurgelun, M.B., Kulke, M.H., Fuchs, C.S., Allen, B.A., Uno, H., Hornick, J.L., Ukaegbu, C.I., Brais, L.K., McNamara, P.G., Mayer, R.J. *et al.* (2017) Cancer susceptibility gene mutations in individuals with colorectal cancer. *J. Clin. Oncol.*, **35**, 1086–1095.
137. Kraus, C., Rau, T.T., Lux, P., Erlenbach-Wünsch, K., Löhr, S., Krumbiegel, M., Thiel, C.T., Stöhr, R., Agaimy, A., Croner, R.S. *et al.* (2015) Comprehensive screening for mutations associated with colorectal cancer in unselected cases reveals penetrant and nonpenetrant mutations. *Int. J. Cancer*, **136**, E559–E568.
138. Cragun, D., Radford, C., Dolinsky, J.S., Caldwell, M., Chao, E. and Pal, T. (2014) Panel-based testing for inherited colorectal cancer: a descriptive study of clinical testing performed by a US laboratory. *Clin. Genet.*, **86**, 510–520.
139. Hansen, M.F., Johansen, J., Sylvander, A.E., Bjørnevoll, I., Talseth-Palmer, B.A., Lavik, L.A.S., Xavier, A., Engebretsen, L.F., Scott, R.J., Drabløs, F. *et al.* (2017) Use of multigene-panel identifies pathogenic variants in several CRC-predisposing genes in patients previously tested for Lynch syndrome. *Clin. Genet.*, **92**, 405–414.
140. Rohlin, A., Rambech, E., Kvist, A., Törngren, T., Eiengård, F., Lundstam, U., Zagoras, T., Gebre-Medhin, S., Borg, Å., Björk, J. *et al.* (2017) Expanding the genotype-phenotype spectrum in hereditary colorectal cancer by gene panel testing. *Fam. Cancer*, **16**, 195–203.
141. Martin-Morales, L., Rofes, P., Diaz-Rubio, E., Llovet, P., Lorca, V., Bando, I., Perez-Segura, P., de la Hoya, M., Garre, P., Garcia-Barberan, V. *et al.* (2018) Novel genetic mutations detected by multigene panel are associated with hereditary colorectal cancer predisposition. *PLoS One*, **13**, e0203885.
142. LaDuca, H., Stuenkel, A.J., Dolinsky, J.S., Keiles, S., Tandy, S., Pesaran, T., Chen, E., Gau, C.-L., Palmaer, E., Shoaepour, K. *et al.* (2014) Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients. *Genet. Med.*, **16**, 830–837.
143. Gong, R., He, Y., Liu, X.-Y., Wang, H.-Y., Sun, L.-Y., Yang, X.-H., Li, B., Cao, X.-K., Ye, Z.-L., Kong, L.-H. *et al.* (2019) Mutation spectrum of germline cancer susceptibility genes among unselected Chinese colorectal cancer patients. *Cancer Manag. Res.*, **11**, 3721–3739.
144. Uson, P.L.S., Jr., Riegert-Johnson, D., Boardman, L., Kisiel, J., Mountjoy, L., Patel, N., Lizaola-Mayo, B., Borad, M.J., Ahn, D., Sonbol, M.B. *et al.* (2021) Germline cancer susceptibility gene testing in unselected patients with colorectal adenocarcinoma: a multicenter prospective study. *Clin. Gastroenterol. Hepatol.*, S1542-3565(21)00447-X.
145. Yurgelun, M.B., Allen, B., Kaldate, R.R., Bowles, K.R., Judkins, T., Kaushik, P., Roa, B.B., Wenstrup, R.J., Hartman, A.-R. and Syngal, S. (2015) Identification of a variety of mutations in cancer predisposition genes in patients with suspected Lynch syndrome. *Gastroenterology*, **149**, 604–13.e20.
146. Susswein, L.R., Marshall, M.L., Nusbaum, R., Vogel Postula, K.J., Weissman, S.M., Yackowski, L., Vaccari, E.M., Bissonnette, J., Booker, J.K., Cremona, M.L. *et al.* (2016) Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. *Genet. Med.*, **18**, 823–832.
147. Fujita, M., Liu, X., Iwasaki, Y., Terao, C., Mizukami, K., Kawakami, E., Takata, S., Inai, C., Aoi, T., Mizukoshi, M. *et al.* (2020) Population-based screening for hereditary colorectal cancer variants in Japan. *Clin. Gastroenterol. Hepatol.*, S1542-3565(20)31664-5.
148. Lorans, M., Dow, E., Macrae, F.A., Winship, I.M. and Buchanan, D.D. (2018) Update on hereditary colorectal cancer: improving the clinical utility of multigene panel testing. *Clin. Colorectal Cancer*, **17**, e293–e305.
149. Heald, B., the Collaborative Group of the Americas on Inherited Gastrointestinal Cancer, Hampel, H., Church, J., Dudley, B., Hall, M.J., Mork, M.E., Singh, A., Stoffel, E., Stoll, J. *et al.* (2020) Collaborative Group of the Americas on Inherited Gastrointestinal Cancer Position statement on multigene panel testing for patients with colorectal cancer and/or polyposis. *Fam. Cancer*, **19**, 223–239.

150. Saeterdal, I., Bjørheim, J., Lislerud, K., Gjertsen, M.K., Bukholm, I.K., Olsen, O.C., Nesland, J.M., Eriksen, J.A., Møller, M., Lindblom, A. *et al.* (2001) Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer. *Proc. Natl. Acad. Sci. U. S. A.*, **98**, 13255–13260.
151. Linnebacher, M., Gebert, J., Rudy, W., Woerner, S., Yuan, Y.P., Bork, P. and von Knebel Doeberitz, M. (2001) Frameshift peptide-derived T-cell epitopes: a source of novel tumor-specific antigens. *Int. J. Cancer*, **93**, 6–11.
152. Tougeron, D., Fauquembergue, E., Rouquette, A., Le Pessot, F., Sesboüé, R., Laurent, M., Berthet, P., Mauillon, J., Di Fiore, F., Sabourin, J.-C. *et al.* (2009) Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. *Mod. Pathol.*, **22**, 1186–1195.
153. Williams, D.S., Bird, M.J., Jorissen, R.N., Yu, Y.L., Walker, F., Zhang, H.H., Nice, E.C. and Burgess, A.W. (2010) Nonsense mediated decay resistant mutations are a source of expressed mutant proteins in colon cancer cell lines with microsatellite instability. *PLoS One*, **5**, e16012.
154. Mlecnik, B., Bindea, G., Angell, H.K., Maby, P., Angelova, M., Tougeron, D., Church, S.E., Lafontaine, L., Fischer, M., Fredriksen, T. *et al.* (2016) Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. *Immunity*, **44**, 698–711.
155. Liu, G.-C., Liu, R.-Y., Yan, J.-P., An, X., Jiang, W., Ling, Y.-H., Chen, J.-W., Bei, J.-X., Zuo, X.-Y., Cai, M.-Y. *et al.* (2018) The heterogeneity between Lynch-associated and sporadic MMR deficiency in colorectal cancers. *J. Natl. Cancer Inst.*, **110**, 975–984.
156. Ballhausen, A., Przybilla, M.J., Jendrusch, M., Haupt, S., Pfaffendorf, E., Seidler, F., Witt, J., Hernandez Sanchez, A., Urban, K., Draxlbauer, M. *et al.* (2020) The shared frameshift mutation landscape of microsatellite-unstable cancers suggests immunoediting during tumor evolution. *Nat. Commun.*, **11**, 4740.
157. Maby, P., Tougeron, D., Hamieh, M., Mlecnik, B., Kora, H., Bindea, G., Angell, H.K., Fredriksen, T., Elie, N., Fauquembergue, E. *et al.* (2015) Correlation between density of CD8 T-cell infiltrate in microsatellite unstable colorectal cancers and frameshift mutations: a rationale for personalized immunotherapy. *Cancer Res.*, **75**, 3446–3455.
158. Schwitalle, Y., Kloor, M., Eiermann, S., Linnebacher, M., Kienle, P., Knaebel, H.P., Tariverdian, M., Benner, A. and von Knebel Doeberitz, M. (2008) Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology*, **134**, 988–997.
159. Kim, H., Jen, J., Vogelstein, B. and Hamilton, S.R. (1994) Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am. J. Pathol.*, **145**, 148–156.
160. Smyrk, T.C., Watson, P., Kaul, K. and Lynch, H.T. (2001) Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. *Cancer*, **91**, 2417–2422.
161. Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pagès, C., Tosolini, M., Camus, M., Berger, A., Wind, P. *et al.* (2006) Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*, **313**, 1960–1964.
162. Popat, S., Hubner, R. and Houlston, R.S. (2005) Systematic review of microsatellite instability and colorectal cancer prognosis. *J. Clin. Oncol.*, **23**, 609–618.
163. Llosa, N.J., Cruise, M., Tam, A., Wicks, E.C., Hechenbleikner, E.M., Taube, J.M., Blosser, R.L., Fan, H., Wang, H., Luber, B.S. *et al.* (2015) The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov.*, **5**, 43–51.
164. Xiao, Y. and Freeman, G.J. (2015) The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. *Cancer Discov.*, **5**, 16–18.
165. Brahmer, J.R., Drake, C.G., Wollner, I., Powderly, J.D., Picus, J., Sharfman, W.H., Stankevich, E., Pons, A., Salay, T.M., McMiller, T.L. *et al.* (2010) Phase I study of single-agent anti–programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J. Clin. Oncol.*, **28**, 3167–3175.
166. Le, D.T., Uram, J.N., Wang, H., Bartlett, B.R., Kemberling, H., Eyring, A.D., Skora, A.D., Luber, B.S., Azad, N.S., Laheru, D. *et al.* (2015) PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.*, **372**, 2509–2520.
167. Le, D.T., Durham, J.N., Smith, K.N., Wang, H., Bartlett, B.R., Aulakh, L.K., Lu, S., Kemberling, H., Wilt, C., Luber, B.S. *et al.* (2017) Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*, **357**, 409–413.
168. Overman, M.J., McDermott, R., Leach, J.L., Lonardi, S., Lenz, H.-J., Morse, M.A., Desai, J., Hill, A., Axelson, M., Moss, R.A. *et al.* (2017) Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol.*, **18**, 1182–1191.
169. Overman, M.J., Lonardi, S., Wong, K.Y.M., Lenz, H.-J., Gelsomino, F., Aglietta, M., Morse, M.A., Van Cutsem, E., McDermott, R., Hill, A. *et al.* (2018) Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J. Clin. Oncol.*, **36**, 773–779.
170. Marabelle, A., Le, D.T., Ascierto, P.A., Di Giacomo, A.M., De Jesus-Acosta, A., Delord, J.-P., Geva, R., Gottfried, M., Penel, N., Hansen, A.R. *et al.* (2020) Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. *J. Clin. Oncol.*, **38**, 1–10.
171. Le, D.T., Kim, T.W., Van Cutsem, E., Geva, R., Jäger, D., Hara, H., Burge, M., O'Neil, B., Kavan, P., Yoshino, T. *et al.* (2020) Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J. Clin. Oncol.*, **38**, 11–19.
172. Wang, F., Zhao, Q., Wang, Y.-N., Jin, Y., He, M.-M., Liu, Z.-X. and Xu, R.-H. (2019) Evaluation of POLE and POLD1 mutations as biomarkers for immunotherapy outcomes across multiple cancer types. *JAMA Oncol.*, **5**, 1504–1506.
173. Mehnert, J.M., Panda, A., Zhong, H., Hirshfield, K., Damare, S., Lane, K., Sokol, L., Stein, M.N., Rodriguez-Rodriquez, L., Kaufman, H.L. *et al.* (2016) Immune activation and response to pembrolizumab in POLE-mutant endometrial cancer. *J. Clin. Invest.*, **126**, 2334–2340.
174. Kalady, M.F., Kravochuck, S.E., Heald, B., Burke, C.A. and Church, J.M. (2015) Defining the adenoma burden in lynch syndrome. *Dis Colon Rectum*, **58**, 388–392.

175. Roukos, D.H., Katsios, C. and Liakakos, T. (2010) Genotype-phenotype map and molecular networks: a promising solution in overcoming colorectal cancer resistance to targeted treatment. *Expert. Rev. Mol. Diagn.*, **10**, 541–545.
176. Kim, B. and Giardiello, F.M. (2011) Chemoprevention in familial adenomatous polyposis. *Best Pract. Res. Clin. Gastroenterol.*, **25**, 607–622.
177. Lee, H.-J., Wang, N.X., Shi, D.-L. and Zheng, J.J. (2009) Sulindac inhibits canonical Wnt signaling by blocking the PDZ domain of the protein dishevelled. *Angew. Chem. Int. Ed. Engl.*, **48**, 6448–6452.
178. Egashira, I., Takahashi-Yanaga, F., Nishida, R., Arioka, M., Igawa, K., Tomooka, K., Nakatsu, Y., Tsuzuki, T., Nakabeppu, Y., Kitazono, T. *et al.* (2017) Celecoxib and 2,5-dimethylcelecoxib inhibit intestinal cancer growth by suppressing the Wnt/β-catenin signaling pathway. *Cancer Sci.*, **108**, 108–115.
179. Kloor, M., Reuschenbach, M., Pauligk, C., Karbach, J., Rafiyan, M.-R., Al-Batran, S.-E., Tariverdian, M., Jäger, E. and von Knebel Doeberitz, M. (2020) A frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers: a phase I/IIa clinical trial. *Clin. Cancer Res.*, **26**, 4503–4510.
180. Risio, M., Reato, G., di Celle, P.F., Fizzotti, M., Rossini, F.P. and Foà, R. (1996) Microsatellite instability is associated with the histological features of the tumor in nonfamilial colorectal cancer. *Cancer Res.*, **56**, 5470–5474.
181. Bernal, M., García-Alcalde, F., Concha, A., Cano, C., Blanco, A., Garrido, F. and Ruiz-Cabello, F. (2012) Genome-wide differential genetic profiling characterizes colorectal cancers with genetic instability and specific routes to HLA class I loss and immune escape. *Cancer Immunol. Immunother.*, **61**, 803–816.
182. Kloor, M., Becker, C., Benner, A., Woerner, S.M., Gebert, J., Ferrone, S. and von Knebel Doeberitz, M. (2005) Immunoselective pressure and human leukocyte antigen class I antigen machinery defects in microsatellite unstable colorectal cancers. *Cancer Res.*, **65**, 6418–6424.
183. Tikidzhieva, A., Benner, A., Michel, S., Formentini, A., Link, K.-H., Dippold, W., von Knebel Doeberitz, M., Kornmann, M. and Kloor, M. (2012) Microsatellite instability and Beta2-microglobulin mutations as prognostic markers in colon cancer: results of the FOGT-4 trial. *Br. J. Cancer*, **106**, 1239–1245.
184. Kloor, M., Michel, S., Buckowitz, B., Rüschoff, J., Büttner, R., Holinski-Feder, E., Dippold, W., Wagner, R., Tariverdian, M., Benner, A. *et al.* (2007) Beta2-microglobulin mutations in microsatellite unstable colorectal tumors. *Int. J. Cancer*, **121**, 454–458.
185. Koelzer, V.H., Baker, K., Kassahn, D., Baumhoer, D. and Zlobec, I. (2012) Prognostic impact of β-2-microglobulin expression in colorectal cancers stratified by mismatch repair status. *J. Clin. Pathol.*, **65**, 996–1002.
186. Pfeifer, G.P. and Besaratinia, A. (2009) Mutational spectra of human cancer. *Hum. Genet.*, **125**, 493–506.
187. Alexandrov, L.B., Nik-Zainal, S., Wedge, D.C., Aparicio, S.A.J.R., Behjati, S., Biankin, A.V., Bignell, G.R., Bolli, N., Borg, A., Børresen-Dale, A.-L. *et al.* (2013) Signatures of mutational processes in human cancer. *Nature*, **500**, 415–421.
188. Alexandrov, L.B., Kim, J., Haradhvala, N.J., Huang, M.N., Tian Ng, A.W., Wu, Y., Boot, A., Covington, K.R., Gordenin, D.A., Bergstrom, E.N. *et al.* (2020) The repertoire of mutational signatures in human cancer. *Nature*, **578**, 94–101.
189. Haradhvala, N.J., Kim, J., Maruvka, Y.E., Polak, P., Rosebrock, D., Livitz, D., Hess, J.M., Leshchiner, I., Kamburov, A., Mouw, K.W. *et al.* (2018) Distinct mutational signatures characterize concurrent loss of polymerase proofreading and mismatch repair. *Nat. Commun.*, **9**, 1746.
190. ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium (2020) Pan-cancer analysis of whole genomes. *Nature*, **578**, 82–93.
191. Van Hoeck, A., Tjoonk, N.H., van Boxtel, R. and Cuppen, E. (2019) Portrait of a cancer: mutational signature analyses for cancer diagnostics. *BMC Cancer*, **19**, 457.
192. Tsui, D.W.Y., Cheng, M.L., Shady, M., Yang, J.L., Stephens, D., Won, H., Srinivasan, P., Huberman, K., Meng, F., Jing, X. *et al.* (2021) Tumor fraction-guided cell-free DNA profiling in metastatic solid tumor patients. *Genome Med.*, **13**, 96.
193. Bettegowda, C., Sausen, M., Leary, R.J., Kinde, I., Wang, Y., Agrawal, N., Bartlett, B.R., Wang, H., Luber, B., Alani, R.M. *et al.* (2014) Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci. Transl. Med.*, **6**, 224ra24.
194. Shen, S.Y., Singhania, R., Fehringer, G., Chakravarthy, A., Roehrl, M.H.A., Chadwick, D., Zuzarte, P.C., Borgida, A., Wang, T.T., Li, T. *et al.* (2018) Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature*, **563**, 579–583.
195. Liu, M.C., Oxnard, G.R., Klein, E.A., Swanton, C., Seiden, M.V. and CCGA Consortium (2020) Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. *Ann. Oncol.*, **31**, 745–759.
196. Willis, J., Lefterova, M.I., Artyomenko, A., Kasi, P.M., Nakamura, Y., Mody, K., Catenacci, D.V.T., Fakih, M., Barbacioru, C., Zhao, J. *et al.* (2019) Validation of microsatellite instability detection using a comprehensive plasma-based genotyping panel. *Clin. Cancer Res.*, **25**, 7035–7045.
197. Kong, S.L., Liu, X., Suhaimi, N.-A.M., Koh, K.J.H., Hu, M., Lee, D.Y.S., Cima, I., Phyo, W.M., Lee, E.X.W., Tai, J.A. *et al.* (2017) Molecular characterization of circulating colorectal tumor cells defines genetic signatures for individualized cancer care. *Oncotarget*, **8**, 68026–68037.
198. Mariotto, A.B., Warren, J.L., Zeruto, C., Coughlan, D., Barrett, M.J., Zhao, L. and Yabroff, K.R. (2020) Cancer-attributable medical costs for colorectal cancer patients by phases of care: what is the effect of a prior cancer history? *J. Natl. Cancer Inst. Monogr.*, **2020**, 22–30.
199. Paszat, L., Sutradhar, R., Luo, J., Rabeneck, L., Tinmouth, J. and Baxter, N.N. (2021) Overall health care cost during the year following diagnosis of colorectal cancer stratified by history of colorectal evaluative procedures. *J.Can. Assoc. Gastroenterol*, gwab001.
200. Schilsky, R.L. (2010) Personalized medicine in oncology: the future is now. *Nat. Rev. Drug Discov.*, **9**, 363–366.
201. Järvinen, H.J. (1992) Epidemiology of familial adenomatous polyposis in Finland: impact of family screening on the colorectal cancer rate and survival. *Gut*, **33**, 357–360.
202. Half, E., Bercovich, D. and Rozen, P. (2009) Familial adenomatous polyposis. *Orphanet J. Rare Dis.*, **4**, 22.
203. InSiGHT. (2016) *InSiGHT*. https://www.insight-group.org/ (accessed Jun 30, 2021).
204. Hamzaoui, N., Alarcon, F., Leulliot, N., Guimbaud, R., Buecher, B., Colas, C., Corsini, C., Nuel, G., Terris, B., Laurent-Puig, P. *et al.* (2020) Genetic, structural, and functional characterization of POLE polymerase proofreading variants allows cancer risk prediction. *Genet. Med.*, **22**, 1533–1541.

205. Kuiper, R.P., Nielsen, M., De Voer, R.M. and Hoogerbrugge, N. (2020) NTHL1 tumor syndrome. In *GeneReviews® [Internet]*. University of Washington, Seattle.
206. Nielsen, M., Infante, E. and Brand, R. (2012) Polyposis. In Adam, M.P., Ardinger, H.H., Pagon, R.A. *et al.* (eds), *GeneReviews*. University of Washington, Seattle, Seattle (WA).
207. Yehia, L. and Eng, C. (2001) Hamartoma tumor syndrome. In Adam, M.P., Ardinger, H.H., Pagon, R.A. *et al.* (eds), *GeneReviews*. University of Washington, Seattle, Seattle (WA).
208. Jasperson, K.W., Tuohy, T.M., Neklason, D.W. and Burt, R.W. (2010) Hereditary and familial colon cancer. *Gastroenterology*, **138**, 2044–2058.
209. American Cancer Society. *American Cancer Society*. https://www.cancer.org (accessed Jun 30, 2021).
210. Shiovitz, S., Copeland, W.K., Passarelli, M.N., Burnett-Hartman, A.N., Grady, W.M., Potter, J.D., Gallinger, S., Buchanan, D.D., Rosty, C., Win, A.K. *et al.* (2014) Characterisation of familial colorectal cancer type X, Lynch syndrome, and non-familial colorectal cancer. *Br. J. Cancer*, **111**, 598–602.
211. Steinke, V., Holzapfel, S., Loeffler, M., Holinski-Feder, E., Morak, M., Schackert, H.K., Görgens, H., Pox, C., Royer-Pokora, B., von Knebel-Doeberitz, M. *et al.* (2014) Evaluating the performance of clinical criteria for predicting mismatch repair gene mutations in Lynch syndrome: a comprehensive analysis of 3,671 families. *Int. J. Cancer*, **135**, 69–77.
212. Antelo, M., Golubicki, M., Roca, E., Mendez, G., Carballido, M., Iseas, S., Cuatrecasas, M., Moreira, L., Sanchez, A., Carballal, S. *et al.* (2019) Lynch-like syndrome is as frequent as Lynch syndrome in early-onset nonfamilial nonpolyposis colorectal cancer. *Int. J. Cancer*, **145**, 705–713.
213. Chika, N., Eguchi, H., Kumamoto, K., Suzuki, O., Ishibashi, K., Tachikawa, T., Akagi, K., Tamaru, J.-I., Okazaki, Y. and Ishida, H. (2017) Prevalence of Lynch syndrome and Lynch-like syndrome among patients with colorectal cancer in a Japanese hospital-based population. *Jpn. J. Clin. Oncol.*, **47**, 108–117.
214. Jung, B., Staudacher, J.J. and Beauchamp, D. (2017) Transforming growth factor β superfamily signaling in development of colorectal cancer. *Gastroenterology*, **152**, 36–52.
215. Salvatore, L., Calegari, M.A., Loupakis, F., Fassan, M., Di Stefano, B., Bensi, M., Bria, E. and Tortora, G. (2019) PTEN in colorectal cancer: shedding light on its role as predictor and target. *Cancer*, **11**, 1765.
216. Liu, D., Keijzers, G. and Rasmussen, L.J. (2017) DNA mismatch repair and its many roles in eukaryotic cells. *Mutat. Res.*, **773**, 174–187.
217. Zhan, T., Rindtorff, N. and Boutros, M. (2017) Wnt signaling in cancer. *Oncogene*, **36**, 1461–1473.
218. Beard, W.A., Horton, J.K., Prasad, R. and Wilson, S.H. (2019) Eukaryotic base excision repair: new approaches shine light on mechanism. *Annu. Rev. Biochem.*, **88**, 137–162.
219. Pavlov, Y.I., Zhuk, A.S. and Stepchenkova, E.I. (2020) DNA polymerases at the eukaryotic replication fork thirty years after: connection to cancer. *Cancer*, **12**, 3489.
220. Shackelford, D.B. and Shaw, R.J. (2009) The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat. Rev. Cancer*, **9**, 563–575.
221. Nishisho, I., Nakamura, Y., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., Koyama, K., Utsunomiya, J., Baba, S. and Hedge, P. (1991) Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science*, **253**, 665–669.
222. Joslyn, G., Carlson, M., Thliveris, A., Albertsen, H., Gelbert, L., Samowitz, W., Groden, J., Stevens, J., Spirio, L. and Robertson, M. (1991) Identification of deletion mutations and three new genes at the familial polyposis locus. *Cell*, **66**, 601–613.
223. Nieminen, T.T., Abdel-Rahman, W.M., Ristimäki, A., Lappalainen, M., Lahermo, P., Mecklin, J.-P., Järvinen, H.J. and Peltomäki, P. (2011) BMPR1A mutations in hereditary nonpolyposis colorectal cancer without mismatch repair deficiency. *Gastroenterology*, **141**, e23–e26.
224. Bellido, F., Sowada, N., Mur, P., Lázaro, C., Pons, T., Valdés-Mas, R., Pineda, M., Aiza, G., Iglesias, S., Soto, J.L. *et al.* (2018) Association between germline mutations in BRF1, a subunit of the RNA polymerase III transcription complex, and hereditary colorectal cancer. *Gastroenterology*, **154**, 181–194.e20.
225. Borck, G., Hög, F., Dentici, M.L., Tan, P.L., Sowada, N., Medeira, A., Gueneau, L., Thiele, H., Kousi, M., Lepri, F. *et al.* (2015) BRF1 mutations alter RNA polymerase III-dependent transcription and cause neurodevelopmental anomalies. *Genome Res.*, **25**, 155–166.
226. Chan, T.L., Yuen, S.T., Kong, C.K., Chan, Y.W., Chan, A.S.Y., Ng, W.F., Tsui, W.Y., Lo, M.W.S., Tam, W.Y., Li, V.S.W. *et al.* (2006) Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. *Nat. Genet.*, **38**, 1178–1183.
227. Ligtenberg, M.J.L., Kuiper, R.P., Chan, T.L., Goossens, M., Hebeda, K.M., Voorendt, M., Lee, T.Y.H., Bodmer, D., Hoenselaar, E., Hendriks-Cornelissen, S.J.B. *et al.* (2009) Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. *Nat. Genet.*, **41**, 112–117.
228. Sivagnanam, M., Mueller, J.L., Lee, H., Chen, Z., Nelson, S.F., Turner, D., Zlotkin, S.H., Pencharz, P.B., Ngan, B.-Y., Libiger, O. *et al.* (2008) Identification of EpCAM as the gene for congenital tufting enteropathy. *Gastroenterology*, **135**, 429–437.
229. Troelstra, C., van Gool, A., de Wit, J., Vermeulen, W., Bootsma, D. and Hoeijmakers, J.H. (1992) ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell*, **71**, 939–953.
230. Mallery, D.L., Tanganelli, B., Colella, S., Steingrimsdottir, H., van Gool, A.J., Troelstra, C., Stefanini, M. and Lehmann, A.R. (1998) Molecular analysis of mutations in the CSB (ERCC6) gene in patients with Cockayne syndrome. *Am. J. Hum. Genet.*, **62**, 77–85.
231. Bonjoch, L., Franch-Expósito, S., Garre, P., Belhadj, S., Muñoz, J., Arnau-Collell, C., Díaz-Gay, M., Gratacós-Mulleras, A., Raimondi, G., Esteban-Jurado, C. *et al.* (2020) Germline mutations in FAF1 are associated with hereditary colorectal cancer. *Gastroenterology*, **159**, 227–240.e7.
232. Seguí, N., Mina, L.B., Lázaro, C., Sanz-Pamplona, R., Pons, T., Navarro, M., Bellido, F., López-Doriga, A., Valdés-Mas, R., Pineda, M. *et al.* (2015) Germline mutations in FAN1 cause hereditary colorectal cancer by impairing DNA repair. *Gastroenterology*, **149**, 563–566.
233. Zhou, W., Otto, E.A., Cluckey, A., Airik, R., Hurd, T.W., Chaki, M., Diaz, K., Lach, F.P., Bennett, G.R., Gee, H.Y. *et al.* (2012) FAN1 mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair. *Nat. Genet.*, **44**, 910–915.
234. Guda, K., Moinova, H., He, J., Jamison, O., Ravi, L., Natale, L., Lutterbaugh, J., Lawrence, E., Lewis, S., Willson, J.K.V. *et al.* (2009) Inactivating germ-line and somatic mutations in polypeptide N-acetylgalactosaminyltransferase 12 in human colon cancers. *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 12921–12925.

235. Jaeger, E., Leedham, S., Lewis, A., Segditsas, S., Becker, M., Cuadrado, P.R., Davis, H., Kaur, K., Heinimann, K., Howarth, K. *et al.* (2012) Hereditary mixed polyposis syndrome is caused by a 40-kb upstream duplication that leads to increased and ectopic expression of the BMP antagonist GREM1. *Nat. Genet.*, **44**, 699–703.
236. Sanders, M.A., Chew, E., Flensburg, C., Zeilemaker, A., Miller, S.E., Al Hinai, A.S., Bajel, A., Luiken, B., Rijken, M., Mclennan, T. *et al.* (2018) MBD4 guards against methylation damage and germ line deficiency predisposes to clonal hematopoiesis and early-onset AML. *Blood*, **132**, 1526–1534.
237. Palles, C., Chew, E., Grolleman, J.E., Galavotti, S., Flensburg, C., Jansen, E.A.M., Curley, H., Chegwidden, L., Barnes, E.A., Bajel, A. *et al.* (2021) Germline loss-of-function variants in the base-excision repair gene MBD4 cause a Mendelian recessive syndrome of adenomatous colorectal polyposis and acute myeloid leukaemia. *BioRxiv* preprint, April 28, 2021. 10.1101/2021.04.27.441137 preprint: not peer reviewed.
238. Wu, Y., Berends, M.J., Sijmons, R.H., Mensink, R.G., Verlind, E., Kooi, K.A., van der Sluis, T., Kempinga, C. and van dDer Zee, A. G., Hollema, H., et al. (2001) A role for MLH3 in hereditary nonpolyposis colorectal cancer. *Nat. Genet.*, **29**, 137–138.
239. Chubb, D., Broderick, P., Dobbins, S.E., Frampton, M., Kinnersley, B., Penegar, S., Price, A., Ma, Y.P., Sherborne, A.L., Palles, C. *et al.* (2016) Rare disruptive mutations and their contribution to the heritable risk of colorectal cancer. *Nat. Commun.*, **7**, 11883.
240. Stewart, G.S., Maser, R.S., Stankovic, T., Bressan, D.A., Kaplan, M.I., Jaspers, N.G., Raams, A., Byrd, P.J., Petrini, J.H. and Taylor, A.M. (1999) The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell*, **99**, 577–587.
241. Nicolaides, N.C., Papadopoulos, N., Liu, B., Wei, Y.F., Carter, K.C., Ruben, S.M., Rosen, C.A., Haseltine, W.A., Fleischmann, R.D. and Fraser, C.M. (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature*, **371**, 75–80.
242. Spier, I., Holzapfel, S., Altmüller, J., Zhao, B., Horpaopan, S., Vogt, S., Chen, S., Morak, M., Raeder, S., Kayser, K. *et al.* (2015) Frequency and phenotypic spectrum of germline mutations in POLE and seven other polymerase genes in 266 patients with colorectal adenomas and carcinomas. *Int. J. Cancer*, **137**, 320–331.
243. Robles-Espinoza, C.D., Harland, M., Ramsay, A.J., Aoude, L.G., Quesada, V., Ding, Z., Pooley, K.A., Pritchard, A.L., Tiffen, J.C., Petljak, M. *et al.* (2014) POT1 loss-of-function variants predispose to familial melanoma. *Nat. Genet.*, **46**, 478–481.
244. Shi, J., Yang, X.R., Ballew, B., Rotunno, M., Calista, D., Fargnoli, M.C., Ghiorzo, P., Bressac-de Paillerets, B., Nagore, E., Avril, M.F. *et al.* (2014) Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. *Nat. Genet.*, **46**, 482–486.
245. Bainbridge, M.N., Armstrong, G.N., Gramatges, M.M., Bertuch, A.A., Jhangiani, S.N., Doddapaneni, H., Lewis, L., Tombrello, J., Tsavachidis, S., Liu, Y. *et al.* (2015) Germline mutations in shelterin complex genes are associated with familial glioma. *J. Natl. Cancer Inst.*, **107**, 384.
246. Calvete, O., Martinez, P., Garcia-Pavia, P., Benitez-Buelga, C., Paumard-Hernández, B., Fernandez, V., Dominguez, F., Salas, C., Romero-Laorden, N., Garcia-Donas, J. *et al.* (2015) A mutation in the POT1 gene is responsible for cardiac angiosarcoma in TP53-negative Li-Fraumeni-like families. *Nat. Commun.*, **6**, 8383.
247. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R. *et al.* (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science*, **275**, 1943–1947.
248. Gala, M.K., Mizukami, Y., Le, L.P., Moriichi, K., Austin, T., Yamamoto, M., Lauwers, G.Y., Bardeesy, N. and Chung, D.C. (2014) Germline mutations in oncogene-induced senescence pathways are associated with multiple sessile serrated adenomas. *Gastroenterology*, **146**, 520–529.
249. Schulz, E., Klampfl, P., Holzapfel, S., Janecke, A.R., Ulz, P., Renner, W., Kashofer, K., Nojima, S., Leitner, A., Zebisch, A. *et al.* (2014) Germline variants in the SEMA4A gene predispose to familial colorectal cancer type X. *Nat. Commun.*, **5**, 5191.
250. Abid, A., Ismail, M., Mehdi, S.Q. and Khaliq, S. (2006) Identification of novel mutations in the SEMA4A gene associated with retinal degenerative diseases. *J. Med. Genet.*, **43**, 378–381.
251. Yu, C.E., Oshima, J., Fu, Y.H., Wijsman, E.M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S. *et al.* (1996) Positional cloning of the Werner's syndrome gene. *Science*, **272**, 258–262.