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Discovery of genetic defects in unexplained colorectal cancer syndromes

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Chapter 7

Concluding remarks and future perspectives

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In this thesis, underlying genetic causes of unexplained suspected Lynch Syndrome and unexplained colonic adenomatous polyposis are presented. Furthermore, practical guidelines are presented to curate variants detected in *PMS2* in DNA isolated from formalin-fixed paraffin-embedded (FFPE) tissue. Additionally, an assay determining the functional effect of splice site variants using RNA isolated from FFPE is presented, all with the ultimate goal to determine the underlying genetic cause of colorectal cancer syndromes. New CRC susceptibility genes and new underlying mechanisms currently explain a portion of the previously unexplained cases, but still a large part of the seemingly familial colorectal cancers remains unexplained. The newly discovered causes, current achievements of determining other susceptibility factors and future perspectives are discussed in this chapter.

Unexplained suspected Lynch Syndrome

In 2014, a study described that in up to 60% of mismatch repair (MMR)-deficient and/or microsatellite unstable tumors no *MLH1* methylation or germline MMR variants are detected.¹ These patients are referred to as 'suspected Lynch Syndrome' (sLS)¹ or Lynch-Like Syndrome (LLS)² and clinical management of these patients remains difficult. Little is known about the cancer risk for these patients, although one study showed they have a lower risk of cancer than patients from LS families, but higher than those from families with sporadic CRC.² Still, without an exact genetic diagnosis, determining surveillance strategies for patients and their relatives is difficult, and can lead to over- as well as undertreatment of family members, e.g. intensive cancer screening in those without an increased CRC risk.³ Several theories have been suggested as to what could be the cause of the MMR-deficiency in these tumors. Three potential reasons for MMR-deficient and/or MSI-H tumors in sLS patients are discussed here.

Missed MMR variants

The most obvious explanation of MMR-deficiency in sLS tumors is missed variants in one of the MMR genes. Since most diagnostic and research testing only screen the coding regions of the MMR genes, (intronic) variants can be missed. One study shows a previously missed deep-intronic *MSH2* variant resulting in inclusion of a pseudoexon.⁴ However, as we have shown in Chapter 2, sequencing of intronic regions results in a large number of intronic variants of uncertain significance (VUS), and classification of these variants is challenging.⁵ Furthermore, while some of these intronic variants might affect splicing, this is difficult to predict using most splice-site prediction software, because these depend strongly on the presence of a canonical splice site nearby the variant. More experimental data are needed to optimize existing prediction tools for deep intronic variants.⁶⁻⁸ According to the variant classification guidelines created by the InSiGHT (International Society for Gastrointestinal Hereditary Tumors) variant interpretation committee, intronic variants, but also missense variants, silent variants and promoter variants, are automatically classified as Class 3 (uncertain significance), until proof of pathogenicity is delivered.

Another category of missed variants are large genomic rearrangements. Detecting large insertions/deletions (indel) with next-generation sequencing (NGS) has been shown to be challenging, and these indels might be missed in current molecular tumor diagnostics.⁹ Some studies describe previously missed inversions^{10, 11} and rearrangements^{12, 13} in sLS patients.

Other explanations include the presence of variants for which the impact on MMR protein function is presently not clear. For example, many studies have investigated the effect that promoter variants can have on gene expression. One well studied promoter variant, *MLH1* c.-27C>A, has been described to confer a CRC-risk by dominant inheritance of a constitutional *MLH1* epimutation, to co-segregate with CRC in multiple families affected with CRC and to lead to reduced *MLH1* expression.¹⁴⁻¹⁷ While in the past described as pathogenic, it is currently in the LOVD database classified as a VUS because of 'insufficient evidence'. Other known promoter variants are the *MLH1* c.-28A>G/-7C>T and the *MLH1* c.-93G>A variants described to show partial loss of expression (c.-28)¹⁸ and increased CRC risk due to possible epigenetic silencing (-93).¹⁹⁻²¹ The latter, however, was classified as benign by the InSiGHT group in 2013, because of the high minor allele frequency (MAF) in the general population.

Variants of uncertain significance remain a concern and functional assays are needed to assess pathogenicity, especially for rare variants found in only a few families or moderately penetrant variants which do not show complete co-segregation in a family. Laboratory efforts capable of assessing the effect of a VUS on various aspects of MMR protein function are cell-free assays determining mismatch repair activity²²⁻²⁴, cell-based assays showing (lack of) expression in CRC cell lines²⁵⁻²⁷, nuclear localization assays and RNA splicing assays using patient RNA^{28, 29} or minigene splicing assays.^{7, 30, 31} Consensus about pathogenicity of a variant is important for proper clinical management.

Somatic inactivation

A quite prevalent cause of MMR-deficiency in sLS patients appears to be biallelic somatic inactivation of the MMR-genes. Multiple studies (Table 1) have shown somatic inactivation in 11% to 100% of the tested sLS cohort.³²⁻³⁷ Of the 68 patients described with biallelic somatic inactivation of one of the MMR genes in these six studies, 37 tumors (54%) had one somatic variant and additional loss of heterozygosity. Patients presented with colorectal, endometrial or small bowel cancer with a mean age of 54.6 years (range 27 - 81 years). Two studies also tested for variants in the exonuclease domain of *POLE* and *POLD1*^{34, 35}, in the other four an underlying variant in one of these genes cannot be excluded. We, and others, have detected

Table 1: Studies on biallelic somatic inactivation in sLS patients

	No. of sLS patients†	No. of biallelic som. MMR	2 MMR	1MMR/ LOH	Average age of onset (years)
Chika et al ³²	2	2	2	0	75.0
Geurts-Giele et al ³³	40	21	5	16	58.9
Haraldsdottir et al ³⁴	27*	19	11	8	53.8
Jansen et al ³⁵	53*	10	5	5	48.1
Mensenkamp et al ³⁶	25	13	5	8	47.7
Sourrouille et al ³⁷	27	3	3	0	55.0
Total	174	68	31	37	54.6

†Only patients without germline MMR variants and without *MLH1* promoter hypermethylation are shown

*sLS patients were tested for *POLE* and *POLD1* variants, patients with a variant were excluded. 2MMR; two somatic variants, 1MMR/LOH; one variant and loss of heterozygosity (LOH) of the WT-allele.

two somatic variants in patients with a family history of CRC, showing that biallelic somatic inactivation does indeed explain the occurrence of a tumor. However, it cannot explain other occurrences of colon cancer in the family and it could be that another underlying genetic cause was missed.^{35, 36} In some cases, the somatic MMR variants are secondary to another (germline) defect that results in a higher mutational load. This has previously been described in patients with MMR-deficient/MSI-H tumors, found to carry biallelic *MUTYH* variants impairing base excision repair (BER).³⁸⁻⁴² In the studies where further testing was performed on the *MUTYH* mutated/MMR-deficient tumors, somatic MMR variants³⁸ or *MLH1* promoter hypermethylation⁴⁰ explained the MMR-deficiency. The somatic MMR variants were MAP-specific G>T variants, indicating that the impaired BER was the primary defect followed by MMR-deficiency.³⁸ This mechanism of secondary MMR-deficiency is also seen in *POLE/POLD1* tumors. These tumors have a high mutational load, leading to many variants that could possibly inactivate genes. These findings underline the importance of screening for variants in other CRC susceptibility genes in patients with biallelic somatic inactivation of the MMR genes but with a positive family history of CRC, especially if the other family members do not show MMR-deficiency/MSI in the tumor. Another possibility is that these somatic variants are present as mosaic variants in the leukocyte DNA, something that has been (albeit very rarely) described before. Sourrouille et al describes one patient where a somatic *MSH2* variant found in the tumor of the mother (but not in blood) was also detected in leukocyte- and tumor DNA of the affected son.³⁷ No other germinal mosaic MMR variants have been described.

Variants in other CRC susceptibility genes

Recent advances in next-generation sequencing (NGS) and whole exome sequencing (WES) or even whole genome sequencing (WGS) resulted in the detection of additional genes possibly involved in tumorigenesis of sLS tumors. As mentioned before, we (Chapter 3), and others, have shown germline and somatic variants in *POLE* or *POLD1* in MMR-deficient tumors.^{34, 35, 43} Variants in the exonuclease domain (EDM) of these genes encoding for polymerase ϵ and δ respectively, have been shown to result in a high mutational burden, often with a high number of C>A transversions.⁴⁴ In sLS tumors it has been hypothesized that a somatic or germline *POLE/POLD1* variant can result in somatic MMR variants, subsequently resulting in microsatellite instability.^{35, 43} Notably, a recent study shows synergistic increase in mutation rate when a pathogenic *POLD1* variant (*POLD1* R689W) was combined with MMR-deficiency, indicating that the *POLD1* mutator effect results from a high rate of replication errors.⁴⁵ This variant is not located in the *POLD1*-EDM but does show impaired nucleotide selectivity, showing that even variants outside the *POLE/POLD1*-EDM might confer an increased CRC susceptibility.^{45, 46}

Other genes that have been implicated as the underlying cause of suspected Lynch Syndrome tumors are *BRCA1*, *BRCA2*, *MUTYH*, *APC*, *STK11*, *MLH3* and *EXO1*.^{38, 47-49} As mentioned before, homozygous and compound-heterozygous *MUTYH* variants have been described in multiple sLS patients, some of which were shown to have secondary MMR-deficiency through somatic MMR variants or *MLH1* promoter hypermethylation.^{38, 40} *BRCA1*, *BRCA2*, *APC* and *STK11* are known dominant cancer predisposition genes previously described to be involved in hereditary breast cancer, familial adenomatous polyposis and Peutz-Jeghers Syndrome respectively.⁵⁰⁻⁵² While the increased risk of CRC for *BRCA1* and *BRCA2* mutation

carriers is still under debate, some studies indicate an up to five fold increased risk of CRC.⁵³⁻⁵⁸ However, the 'suspected Lynch Syndrome' patients described in these studies found to carry these *BRCA1*, *BRCA2*, *APC* and *STK11* variants were selected based on personal and family history of CRC but no IHC of the four MMR genes or MSI was performed.

MLH3 and *EXO1* are both mismatch repair genes. *MLH3* interacts with *MLH1*, is believed to participate in insertion deletion loop (IDL) repair and has been shown to exhibit MSI in cell culture.^{59, 60} The role of the mismatch repair gene *MLH3* in colorectal tumorigenesis is under debate.⁶¹⁻⁶⁴ Whereas one study describes germline *MLH3* variants in sLS patients⁶¹ (with positive *MLH1*, *MSH2* and *MSH6* staining, but with MSI), more evidence is needed before it can be regarded as a causal (possible reduced penetrant) Lynch Syndrome gene. The exonuclease 1 (*EXO1*) gene encodes a 5' → 3' exonuclease that is involved in multiple DNA repair pathways.^{65, 66} In MMR, *EXO1* interacts with *MSH2* and in yeast it shows a mutator phenotype when lost.⁶⁶ *EXO1* has been extensively studied in CRC^{48, 64, 67-71}, and is often found not to be associated with (suspected) Lynch Syndrome.^{67, 70, 71} Single nucleotide polymorphisms (SNPs) in this gene have been described to confer a slightly increased risk of CRC in the general population, but have not been associated with LS.^{68, 69} Wu et al describes germline *EXO1* variants in 14 patients fulfilling Amsterdam criteria, with six of the tumors showing microsatellite instability.⁴⁸ Twelve of thirteen tested tumors showed loss of heterozygosity with retention of the wildtype allele. The authors suggest that complete loss of *EXO1* is lethal to the cell, and that a haploinsufficiency effect can give rise to tumors. Other studies screened for *EXO1* variants in sLS patients, but no other carriers were found.^{72, 73} This, together with the fact that patients were not screened for variants in established LS/CRC susceptibility genes (*PMS2*, *POLE*, *POLD1*), shows little evidence to include *EXO1* as a LS gene, although a role as a low penetrant cancer susceptibility gene cannot be excluded at this point in time.

For Lynch Syndrome tumorigenesis, it remains unclear when the MMR-deficient phenotype (e.g. loss of the second allele in germline MMR carriers) is acquired during tumorigenesis. Multiple studies tested adenomas from MMR-mutation carriers for immunohistochemical loss of the MMR proteins or MSI and found a Lynch phenotype in 40-80% of tested adenomas, often associated with adenoma size.⁷⁴⁻⁸⁰ Ahadova et al proposed two pathways in Lynch Syndrome (see Figure 1), one where adenomatous polyps precede MMR-deficiency with an initiating event as *APC* and/or *KRAS* gene variants and one where MMR gene inactivation is the initial event leading to non-polypous precursor lesions and secondary *CTNNB1* hits are needed for tumorigenesis.⁸¹ While it can be debated whether polypous growth precedes MMR gene inactivation in LS tumors, this is probably the preferred pathway in suspected Lynch Syndrome patients with somatic MMR inactivation. In these patients the underlying genetic defects (*MUTYH*, *POLE*, *POLD1*) often result in a mutator phenotype, with the MMR-deficiency acting as a secondary defect resulting in tumorigenesis. It is conceivable that for these patients adenomas can arise before mismatch repair is completely inactivated. Immunohistochemical staining and MSI analysis of these tumors gives a bias to expect an MMR defect, while the true underlying germline defect could be missed. Especially when patients present with early onset colorectal cancer and a positive family history of CRC, tumors should be screened for variants in other CRC susceptibility genes. Whole exome sequencing of unexplained suspected Lynch Syndrome patients might detect CRC susceptibility genes

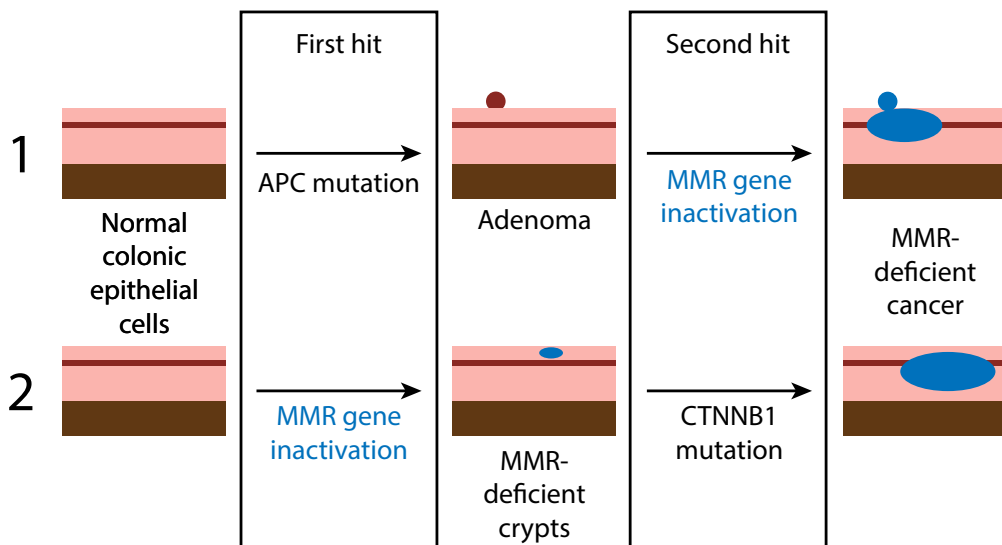


Figure 1: proposed pathways of colorectal tumorigenesis in Lynch Syndrome as described by Ahadova et al, *Familial Cancer*, 2016.⁸¹

or loci which are currently not known, although this may require very large study sizes. The expectation is that through these approaches, rare genetic variants or high-risk combinations of CRC susceptibility SNPs can be detected, an effort that is already being done in MMR-proficient, microsatellite stable tumors.^{82, 83}

Unexplained polyposis

In approximately 76-82% of the severe and typical FAP patients a pathogenic germline variant in *APC* is found, while the majority of patients with atypical or attenuated polyposis can be explained by *APC* or homozygous/compound-heterozygous germline *MUTYH* variants.⁸⁴⁻⁸⁶ Still, in a small fraction of patients no genetic predisposition can be found.

In recent years three new polyposis syndromes have been described which may account for a part of these unexplained polyposis cases. These syndromes are polymerase proofreading associated polyposis (PPAP)⁸⁷, caused by variants in the proofreading domain of *POLE* and *POLD1*, NTHL1-associated polyposis (NAP)⁸⁸, caused by homozygous or compound-heterozygous variants in the base excision repair gene *NTHL1* and the recently described MSH3-associated polyposis⁸⁹ caused by biallelic germline inactivation of the mismatch repair gene *MSH3*. Both PPAP and NAP have a variable phenotype with polyps but also with the occurrence of colorectal cancers and other extracolonic tumors. Both are also described to confer a specific mutation spectrum, with a *POLE/POLD1* defect resulting in an ultramutated phenotype with an increase of C:T>A:G transversions and *NTHL1* deficient tumors showing an increase in C:G>T:A transitions.^{44, 88} Compound-heterozygous *MSH3* variants have only been described in two unrelated individuals, one female with over 40 polyps and a history of thyroid adenoma (age 35), uterine leiomyoma (age 44) and polyps in corpus uteri and

duodenum, and one female with multiple adenomas at age 32, astrocytoma (age 26), ovarian and dermoid cysts and follicular thyroid adenomas at age 42.⁸⁹ Both also had small intraductal papillomas in the mammary gland. Whether these neoplasms are specific for an *MSH3* defect is unknown, and can only be concluded after more *MSH3* variant carriers are detected.

Of all patients carrying a pathogenic *de novo* *APC* variant, explaining the FAP phenotype, approximately 20% are estimated to have somatic mosaicism.⁹⁰⁻⁹² This phenomenon, in which (*APC*) variants are only present in a fraction of the cells, was already described in 1999 in a study where parents of five *de novo* FAP patients were tested for the *APC* variant found in the index patient.⁹³ Multiple studies have been conducted since then, always focusing on testing leukocyte DNA for variants with a low variant allele frequency, or on analyzing parents of *de novo* patients.^{90-92, 94-99} We (Chapter 5) and others have recently shown a high number of missed mosaic *APC* patients, by sequencing multiple adenomas of the same patients.^{100, 101} If two or more adenomas carry an identical *APC* variant, this might indicate an underlying *APC* mosaicism. This strategy has been proven to be more sensitive and specific than sequencing leukocyte DNA for variants with a low variant frequency, and can detect mosaicism confined to the colon.^{100, 101} Early detection of patients with somatic *APC* mosaicism is important and will help guide clinical management. Patients often show an attenuated FAP phenotype but if the variant is inherited by the patients offspring, they will show a full blown FAP phenotype with possibly 100 to more than 1000 adenomas.^{90, 91, 100}

Besides the recently described colorectal cancer syndromes with *POLE*, *POLD1*, *NTHL1* and *MSH3* germline variants, other genes have been implicated in unexplained familial polyposis syndromes. Three recent studies performing whole exome sequencing or genome-wide SNP genotyping in unexplained adenomatous polyposis patients describe variants or copy number variations in the *CNTN6*, *FOCAD*, *HSPH1*, *KIF26B*, *MCM3AP*, *YBEY*, *ARHGAP*, *CTNNB1*, *DSC2*, *PIEZO1*, *ZSWIM7* and *MCM9* genes.¹⁰²⁻¹⁰⁴ Variants in *DSC2*, *PIEZO1* and *ZSWIM7* were first detected in a cohort of 7 unrelated polyposis patients with 20-40 adenomas. Sequencing these three genes in a validation cohort of 191 unexplained polyposis patients resulted in the detection of 4 additional *DSC2* variants and 4 additional *PIEZO1* variants.¹⁰⁴ Copy number variant (CNV) analysis in 221 unexplained polyposis patients showed rare, non-recurrent germline CNVs in 77 proteins.¹⁰³ Targeted NGS found point-mutations in 10 of the 77 investigated genes (*CNTN6*, *FOCAD*, *HSPH1*, *KIF26B*, *MCM3AP*, *YBEY*, *CTNNB1* and three genes from the *ARHGAP* family).¹⁰³ Of these 10 genes, only *FOCAD* and *CTNNB1*, involved in Wnt signaling, have previously been related to CRC or colorectal adenoma predisposition.^{105, 106} The third study shows homozygous variants in the *MCM9* gene in two sisters with multiple polyps and metastatic CRC at young age. The *MCM9* gene encodes a DNA helicase involved in homologous recombination (HR) and mismatch repair (MMR).¹⁰⁷ However, an independent study sequencing *MCM9* in suspected Lynch Syndrome patients only detected variants of uncertain significance.¹⁰⁸ For all newly discovered genes more evidence is needed to establish them as CRC or polyposis susceptibility genes.

Other factors in colorectal cancer

While genetic factors are expected to play an important role in families with multiple affected patients, other factors are known to increase CRC susceptibility. Diet is an important factor, and it has been shown that a Western diet characterized by high intake of meat, refined

sugar and saturated fat, but lacking in fiber, contributes to development of CRC.¹⁰⁹⁻¹¹² Other lifestyle factors contributing to CRC risk are excess body weight, low physical activity, alcohol consumption and smoking.¹¹¹⁻¹¹³ Calcium, fiber, milk and whole grains on the other hand, have been associated with a lower risk of CRC.¹¹²⁻¹¹⁴

Another factor involved in increased CRC is the gut microbiota. The human intestinal tract is inhabited by trillions of microorganisms and the presence of specific bacteria or the dysbiosis of bacteria can aid in development and progression of colorectal cancer.^{115, 116} Bacteria can affect CRC tumorigenesis by secretion of toxins that can induce DNA damage and secretion of metabolites that affect translation, gene regulation and cell proliferation.^{115, 116} The gut microbiome is also shown to affect innate immunity through activation of toll-like receptors and through influencing T-cell differentiation.^{115, 116} Interestingly, diet and alcohol consumption have been shown to be able to dysregulate microbiota, possibly explaining the link between diet and colorectal cancer.¹¹⁵⁻¹¹⁷

Abovementioned non-genetic factors can result in believing multiple cancers in a family are due to an underlying genetic cause, while in fact the affected patients are phenocopies, i.e. displaying characteristics of a certain genotype but produced by environmental factors. This underlines a challenge in determining the underlying genetic cause in unexplained families with CRC. While WES and WGS enable the detection of rare pathogenic variants, it is difficult to determine whether a family with a few affected family members is indeed the result of one dominant DNA variation. Colorectal cancer is a common disease, and many environmental, as well as polygenic, factors can modulate CRC risk. A recent study shows that families with exactly two first-degree relatives only have a moderate probability of being due to segregating familial disease and advises to first focus on families with three or more members to increase the probability of finding genetic factors.¹¹⁸

Final conclusions

The estimated worldwide incidence of colorectal cancer is 746,000 new cases annually.¹¹⁹ Approximately 15% of these colorectal cancers will display MSI, while 3% of these CRCs will carry a germline MMR variant. Routine molecular screening of all early-onset CRCs with immunohistochemical staining of the four MMR proteins and/or MSI analysis yields a high number of suspected Lynch Syndrome cases of which the majority will likely remain suspected after conventional germline mutation screening and MSI analysis. In this thesis we describe possible genetic causes of unexplained suspected Lynch Syndrome (sLS) and unexplained adenomatous polyposis. We hypothesize that unexplained sLS patients could be explained by (1) missed MMR variants (Chapter 2), (2) by biallelic somatic inactivation (Chapter 2 and 3) or (3) by variants in other CRC susceptibility genes (Chapter 3).

We (Chapter 2 and 3), and others, show that biallelic somatic inactivation could possibly explain up to half of all unexplained sLS patients. Screening for somatic MMR variants should be broadly introduced in molecular tumor diagnostics, giving more insight in these sporadic MMR-deficient cases. Families should be critically assessed, so no underlying genetic variants are missed in these seemingly sporadic cases. Recent advances in (whole) exome and targeted next-generation sequencing enable detection of rare variants in previously unknown CRC susceptibility genes, and show that MMR-deficiency could be due to (secondary) somatic events, sometimes with underlying germline gene variants in genes such as *POLE*, *POLD1* or *MUTYH*. In future research it would be interesting to test affected family members of the patients of whom the tumor occurrence is explained by somatic events, to see whether these patients also have MMR-deficient tumors and to find a common underlying genetic cause in these family members.

While missed MMR variants seem a rare event, explaining only a fraction of patients (Chapter 2), it cannot be excluded that the remaining unexplained sLS tumors still carry undetected MMR variants. These variants could be currently misunderstood and classified as uncertain significance (VUS) or could be missed by conventional screening, because they are in intronic regions or in close proximity to the MMR genes. Functional assessment of VUS is critical. In Chapter 4 we show an easy method to detect aberrant splicing in formalin-fixed paraffin-embedded tissue, showing opportunities for the future. However, while this method enables functional assessment of variants predicted to result in splicing, it cannot detect aberrant splicing when the mutation status is unknown. A high-throughput NGS-based assay sequencing all exon-exon boundaries in RNA to detect any possible RNA aberration could possibly detect the cause of the MMR-deficiency. Also, it could indicate missed deep-intronic variants resulting in an aberrant RNA product. Furthermore, the current study relies on *in silico* prediction tools and effects that fall outside the amplification window will not be detected, while a high throughput assay would analyse the RNA in an unbiased manner. Other currently undiscovered mechanisms leading to aberrant RNA could be detected with this unbiased NGS approach.

The introduction of the population-based screening leads to an increasing number of patients with a low number of polyps (5-40) at older age (60+) which would previously go undiscovered. We (Chapter 6), and others, recently showed that many of these patients with

an attenuated FAP (AFAP) phenotype carry somatic mosaic *APC* variants. Current screening consisting of screening leukocyte DNA for variants with a low variant allele frequency is shown to miss many of these patients, because the variant is present with a too low variant allele frequency or because the mosaicism is confined to the colon. In-depth analyses of adenomas of AFAP patients could lead to the detection of more mosaic *APC* carriers, affecting clinical management of their children, who, if the variant is inherited, will show a full blown FAP phenotype.

While the exact incidence of biallelic somatic MMR variants, *POLE/POLD1* variants with secondary MMR-deficiency and somatic mosaicism still needs to be assessed, a large fraction of the previously unexplained sLS patients can now be explained and receive proper clinical management. However, in the remaining, approximately, 20-40% of sLS patients the underlying (familial) cause is still unknown. Whole exome or genome sequencing in the future will possibly lead to the detection of more rare gene variants, variants with moderate increase in CRC susceptibility or variants with moderate penetrance. Joint efforts screening for variants in larger cohorts and data sharing are essential in finding these (low penetrant) CRC susceptibility loci and might enable establishment of a CRC polygenic risk model with a personal cancer risk score.

References

- Buchanan DD, Rosty C, Clendenning M, et al. Clinical problems of colorectal cancer and endometrial cancer cases with unknown cause of tumor mismatch repair deficiency (suspected Lynch syndrome). *Appl Clin Genet* 2014;7:183-93.
- Rodriguez-Soler M, Perez-Carbonell L, Guarinos C, et al. Risk of cancer in cases of suspected lynch syndrome without germline mutation. *Gastroenterology* 2013;144:926-932 e1; quiz e13-4.
- Castells A, Castellvi-Bel S, Balaguer F. Concepts in Familial Colorectal Cancer: Where Do We Stand and What Is the Future? *Gastroenterology*;137:404-409.
- Clendenning M, Buchanan DD, Walsh MD, et al. Mutation deep within an intron of MSH2 causes Lynch syndrome. *Fam Cancer* 2011;10:297-301.
- Jansen AM, Geilenkirchen MA, van Wezel T, et al. Whole Gene Capture Analysis of 15 CRC Susceptibility Genes in Suspected Lynch Syndrome Patients. *PLoS One* 2016;11:e0157381.
- Colombo M, De Vecchi G, Caleca L, et al. Comparative in vitro and in silico analyses of variants in splicing regions of BRCA1 and BRCA2 genes and characterization of novel pathogenic mutations. *PLoS One* 2013;8:e57173.
- van der Klift HM, Jansen AM, van der Steenstraten N, et al. Splicing analysis for exonic and intronic mismatch repair gene variants associated with Lynch syndrome confirms high concordance between minigene assays and patient RNA analyses. *Mol Genet Genomic Med* 2015;3:327-45.
- Vreeswijk MP, Kraan JN, van der Klift HM, et al. Intronic variants in BRCA1 and BRCA2 that affect RNA splicing can be reliably selected by splice-site prediction programs. *Hum Mutat* 2009;30:107-14.
- Ye K, Wang J, Jayasinghe R, et al. Systematic discovery of complex insertions and deletions in human cancers. *Nat Med* 2016;22:97-104.
- Rhees J, Arnold M, Boland CR. Inversion of exons 1-7 of the MSH2 gene is a frequent cause of unexplained Lynch syndrome in one local population. *Fam Cancer* 2014;13:219-25.
- Mork ME, Rodriguez A, Taggart MW, et al. Identification of MSH2 inversion of exons 1-7 in clinical evaluation of families with suspected Lynch syndrome. *Fam Cancer* 2017;16:357-361.
- Perez-Cabornero L, Borrás Flores E, Infante Sanz M, et al. Characterization of new founder Alu-mediated rearrangements in MSH2 gene associated with a Lynch syndrome phenotype. *Cancer Prev Res (Phila)* 2011;4:1546-55.
- Plaschke J, Ruschhoff J, Schackert HK. Genomic rearrangements of hMSH6 contribute to the genetic predisposition in suspected hereditary non-polyposis colorectal cancer syndrome. *J Med Genet* 2003;40:597-600.
- Hitchins MP, Rapkins RW, Kwok CT, et al. Dominantly inherited constitutional epigenetic silencing of MLH1 in a cancer-affected family is linked to a single nucleotide variant within the 5'UTR. *Cancer Cell* 2011;20:200-13.
- Kwok CT, Vogelaar IP, van Zelst-Stams WA, et al. The MLH1 c.-27C>A and c.85G>T variants are linked to dominantly inherited MLH1 epimutation and are borne on a European ancestral haplotype. *Eur J Hum Genet* 2014;22:617-24.
- Ward RL, Dobbins T, Lindor NM, et al. Identification of constitutional MLH1 epimutations and promoter variants in colorectal cancer patients from the Colon Cancer Family Registry. *Genet Med* 2013;15:25-35.
- Raevaara TE, Korhonen MK, Lohi H, et al. Functional significance and clinical phenotype of nontruncating mismatch repair variants of MLH1. *Gastroenterology* 2005;129:537-49.
- Hesson LB, Packham D, Kwok CT, et al. Lynch syndrome associated with two MLH1 promoter variants and allelic imbalance of MLH1 expression. *Hum Mutat* 2015;36:622-30.
- Raptis S, Mrkonjic M, Green RC, et al. MLH1 -93G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. *J Natl Cancer Inst* 2007;99:463-74.
- Wang T, Liu Y, Sima L, et al. Association between MLH1 -93G>a polymorphism and risk of colorectal cancer. *PLoS One* 2012;7:e50449.
- Chen H, Taylor NP, Sotamaa KM, et al. Evidence for heritable predisposition to epigenetic silencing of MLH1. *Int J Cancer* 2007;120:1684-8.
- Drost M, Zonneveld J, van Dijk L, et al. A cell-free assay for the functional analysis of variants of the mismatch repair protein MLH1. *Hum Mutat* 2010;31:247-53.
- Drost M, Zonneveld JB, van Hees S, et al. A rapid and cell-free assay to test the activity of lynch syndrome-associated MSH2 and MSH6 missense variants. *Hum Mutat* 2012;33:488-94.
- Drost M, Koppejan H, de Wind N. Inactivation of DNA mismatch repair by variants of uncertain significance in the PMS2 gene. *Hum Mutat* 2013;34:1477-80.
- Trojan J, Zeuzem S, Randolph A, et al. Functional analysis of hMLH1 variants and HNPCC-related mutations using a human expression system. *Gastroenterology* 2002;122:211-9.

26. Takahashi M, Shimodaira H, Andreutti-Zaugg C, et al. Functional analysis of human MLH1 variants using yeast and in vitro mismatch repair assays. *Cancer Res* 2007;67:4595-604.
27. Perera S, Bapat B. The MLH1 variants p.Arg265Cys and p.Lys618Ala affect protein stability while p.Leu749Gln affects heterodimer formation. *Hum Mutat* 2008;29:332.
28. Sharp A, Pichert G, Lucassen A, et al. RNA analysis reveals splicing mutations and loss of expression defects in MLH1 and BRCA1. *Hum Mutat* 2004;24:272.
29. Pagenstecher C, Wehner M, Friedl W, et al. Aberrant splicing in MLH1 and MSH2 due to exonic and intronic variants. *Hum Genet* 2006;119:9-22.
30. Auclair J, Busine MP, Navarro C, et al. Systematic mRNA analysis for the effect of MLH1 and MSH2 missense and silent mutations on aberrant splicing. *Hum Mutat* 2006;27:145-54.
31. Tournier I, Vezain M, Martins A, et al. A large fraction of unclassified variants of the mismatch repair genes MLH1 and MSH2 is associated with splicing defects. *Hum Mutat* 2008;29:1412-24.
32. Chika N, Eguchi H, Kumamoto K, et al. Prevalence of Lynch syndrome and Lynch-like syndrome among patients with colorectal cancer in a Japanese hospital-based population. *Jpn J Clin Oncol* 2017;47:108-117.
33. Geurts-Giele WR, Leenen CH, Dubbink HJ, et al. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. *J Pathol* 2014;234:548-59.
34. Haraldsdottir S, Hampel H, Tomsic J, et al. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology* 2014;147:1308-1316 e1.
35. Jansen AM, van Wezel T, van den Akker BE, et al. Combined mismatch repair and POLE/POLD1 defects explain unresolved suspected Lynch syndrome cancers. *Eur J Hum Genet* 2016;24:1089-92.
36. Mensenkamp AR, Vogelaar IP, van Zelst-Stams WA, et al. Somatic mutations in MLH1 and MSH2 are a frequent cause of mismatch-repair deficiency in Lynch syndrome-like tumors. *Gastroenterology* 2014;146:643-646 e8.
37. Sourrouille I, Coulet F, Lefevre JH, et al. Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. *Fam Cancer* 2013;12:27-33.
38. Morak M, Heidenreich B, Keller G, et al. Biallelic MUTYH mutations can mimic Lynch syndrome. *Eur J Hum Genet* 2014;22:1334-7.
39. Lefevre JH, Rodrigue CM, Mourra N, et al. Implication of MYH in colorectal polyposis. *Ann Surg* 2006;244:874-9; discussion 879-80.
40. Colebatch A, Hitchins M, Williams R, et al. The role of MYH and microsatellite instability in the development of sporadic colorectal cancer. *Br J Cancer* 2006;95:1239-43.
41. O'Shea AM, Cleary SP, Croitoru MA, et al. Pathological features of colorectal carcinomas in MYH-associated polyposis. *Histopathology* 2008;53:184-94.
42. Cleary SP, Cotterchio M, Jenkins MA, et al. Germline MutY human homologue mutations and colorectal cancer: a multisite case-control study. *Gastroenterology* 2009;136:1251-60.
43. Elsayed FA, Kets CM, Ruano D, et al. Germline variants in POLE are associated with early onset mismatch repair deficient colorectal cancer. *Eur J Hum Genet* 2015;23:1080-4.
44. Shinbrot E, Henninger EE, Weinhold N, et al. Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. *Genome Res* 2014;24:1740-50.
45. Mertz TM, Baranovskiy AG, Wang J, et al. Nucleotide selectivity defect and mutator phenotype conferred by a colon cancer-associated DNA polymerase delta mutation in human cells. *Oncogene* 2017;36:4427-4433.
46. Mertz TM, Sharma S, Chabes A, et al. Colon cancer-associated mutator DNA polymerase delta variant causes expansion of dNTP pools increasing its own infidelity. *Proc Natl Acad Sci U S A* 2015;112:E2467-76.
47. Yurgelun MB, Allen B, Kaldate RR, et al. Identification of a Variety of Mutations in Cancer Predisposition Genes in Patients With Suspected Lynch Syndrome. *Gastroenterology* 2015;149:604-13 e20.
48. Wu Y, Berends MJ, Post JG, et al. Germline mutations of EXO1 gene in patients with hereditary nonpolyposis colorectal cancer (HNPCC) and atypical HNPCC forms. *Gastroenterology* 2001;120:1580-7.
49. Castillejo A, Vargas G, Castillejo MI, et al. Prevalence of germline MUTYH mutations among Lynch-like syndrome patients. *Eur J Cancer* 2014;50:2241-50.
50. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;66:589-600.
51. Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990;250:1684-9.
52. Jenne DE, Reimann H, Nezu J, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nature Genetics* 1998;18:38-44.
53. Thompson D, Easton DF. Cancer Incidence in BRCA1 Mutation Carriers. *JNCI: Journal of the National Cancer Institute* 2002;94:1358-1365.
54. Sopik V, Phelan C, Cybulski C, et al. BRCA1 and BRCA2 mutations and the risk for colorectal cancer. *Clin*

- Genet 2015;87:411-8.
55. Brose MS, Rebbeck TR, Calzone KA, et al. Cancer Risk Estimates for BRCA1 Mutation Carriers Identified in a Risk Evaluation Program. *JNCI: Journal of the National Cancer Institute* 2002;94:1365-1372.
56. Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA1-mutation carriers. *The Lancet* 1994;343:692-695.
57. Phelan CM, Iqbal J, Lynch HT, et al. Incidence of colorectal cancer in BRCA1 and BRCA2 mutation carriers: results from a follow-up study. *Br J Cancer* 2014;110:530-534.
58. Kadouri L, Hubert A, Rotenberg Y, et al. Cancer risks in carriers of the BRCA1/2 Ashkenazi founder mutations. *J Med Genet* 2007;44:467-71.
59. Lipkin SM, Wang V, Jacoby R, et al. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nat Genet* 2000;24:27-35.
60. Chen PC, Kuraguchi M, Velasquez J, et al. Novel roles for MLH3 deficiency and TLE6-like amplification in DNA mismatch repair-deficient gastrointestinal tumorigenesis and progression. *PLoS Genet* 2008;4:e1000092.
61. Wu Y, Berends MJ, Sijmons RH, et al. A role for MLH3 in hereditary nonpolyposis colorectal cancer. *Nat Genet* 2001;29:137-8.
62. Hienonen T, Laiho P, Salovaara R, et al. Little evidence for involvement of MLH3 in colorectal cancer predisposition. *Int J Cancer* 2003;106:292-6.
63. Liu HX, Zhou XL, Liu T, et al. The role of hMLH3 in familial colorectal cancer. *Cancer Res* 2003;63:1894-9.
64. Kim JC, Roh SA, Yoon YS, et al. MLH3 and EXO1 alterations in familial colorectal cancer patients not fulfilling Amsterdam criteria. *Cancer Genet Cytogenet* 2007;176:172-4.
65. Goellner EM, Putnam CD, Kolodner RD. Exonuclease 1-dependent and independent mismatch repair. *DNA Repair (Amst)* 2015;32:24-32.
66. Tishkoff DX, Boerger AL, Bertrand P, et al. Identification and characterization of *Saccharomyces cerevisiae* EXO1, a gene encoding an exonuclease that interacts with MSH2. *Proc Natl Acad Sci U S A* 1997;94:7487-92.
67. Thompson E, Meldrum CJ, Crooks R, et al. Hereditary non-polyposis colorectal cancer and the role of hPMS2 and hEXO1 mutations. *Clin Genet* 2004;65:215-25.
68. Yamamoto H, Hanafusa H, Ouchida M, et al. Single nucleotide polymorphisms in the EXO1 gene and risk of colorectal cancer in a Japanese population. *Carcinogenesis* 2005;26:411-416.
69. Zhang M, Zhao D, Yan C, et al. Associations between Nine Polymorphisms in EXO1 and Cancer Susceptibility: A Systematic Review and Meta-Analysis of 39 Case-control Studies. *Scientific Reports* 2016;6:29270.
70. Jagmohan-Changur S, Poikonen T, Vilkkilä S, et al. EXO1 variants occur commonly in normal population: evidence against a role in hereditary nonpolyposis colorectal cancer. *Cancer Res* 2003;63:154-8.
71. Alam NA, Gorman P, Jaeger EE, et al. Germline deletions of EXO1 do not cause colorectal tumors and lesions which are null for EXO1 do not have microsatellite instability. *Cancer Genet Cytogenet* 2003;147:121-7.
72. Talseth-Palmer BA, Bauer DC, Sijns W, et al. Targeted next-generation sequencing of 22 mismatch repair genes identifies Lynch syndrome families. *Cancer Med* 2016;5:929-41.
73. Chen W, Swanson BJ, Frankel WL. Molecular genetics of microsatellite-unstable colorectal cancer for pathologists. *Diagnostic Pathology* 2017;12:24.
74. Sekine S, Mori T, Ogawa R, et al. Mismatch repair deficiency commonly precedes adenoma formation in Lynch Syndrome-Associated colorectal tumorigenesis. *Mod Pathol* 2017;30:1144-1151.
75. De Jong AE, Morreau H, Van Puijenbroek M, et al. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. *Gastroenterology* 2004;126:42-48.
76. Pino MS, Mino-Kenudson M, Wildmore BM, et al. Deficient DNA Mismatch Repair Is Common in Lynch Syndrome-Associated Colorectal Adenomas. *The Journal of Molecular Diagnostics* 2009;11:238-247.
77. Walsh MD, Buchanan DD, Pearson SA, et al. Immunohistochemical testing of conventional adenomas for loss of expression of mismatch repair proteins in Lynch syndrome mutation carriers: a case series from the Australasian site of the colon cancer family registry. *Mod Pathol* 2012;25:722-30.
78. Yurgelun MB, Goel A, Hornick JL, et al. Microsatellite instability and DNA mismatch repair protein deficiency in Lynch syndrome colorectal polyps. *Cancer Prev Res (Phila)* 2012;5:574-82.
79. Halvarsson B, Lindblom A, Johansson L, et al. Loss of mismatch repair protein immunostaining in colorectal adenomas from patients with hereditary nonpolyposis colorectal cancer. *Mod Pathol* 2005;18:1095-1101.
80. Iino H, Simms L, Young J, et al. DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. *Gut* 2000;47:37-42.
81. Ahadova A, von Knebel Doeberitz M, Blaker H, et al. CTNNB1-mutant colorectal carcinomas with immediate invasive growth: a model of interval cancers in Lynch syndrome. *Fam Cancer* 2016;15:579-86.
82. Ku C-S, Cooper DN, Wu M, et al. Gene discovery in familial cancer syndromes by exome sequencing: prospects for the elucidation of familial colorectal cancer type X. *Mod Pathol* 2012;25:1055-1068.
83. de Voer RM, Hahn MM, Weren RD, et al. Identification of Novel Candidate Genes for Early-Onset Colorectal

- Cancer Susceptibility. *PLoS Genet* 2016;12:e1005880.
84. Nielsen M, Hes FJ, Nagengast FM, et al. Germline mutations in APC and MUTYH are responsible for the majority of families with attenuated familial adenomatous polyposis. *Clin Genet* 2007;71:427-33.
 85. Aretz S, Uhlhaas S, Goergens H, et al. MUTYH-associated polyposis: 70 of 71 patients with biallelic mutations present with an attenuated or atypical phenotype. *Int J Cancer* 2006;119:807-14.
 86. Friedl W, Aretz S. Familial adenomatous polyposis: experience from a study of 1164 unrelated german polypoid patients. *Hered Cancer Clin Pract* 2005;3:95-114.
 87. Palles C, Cazier JB, Howarth KM, et al. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat Genet* 2013;45:136-44.
 88. Weren RD, Ligtenberg MJ, Kets CM, et al. A germline homozygous mutation in the base-excision repair gene NTHL1 causes adenomatous polyposis and colorectal cancer. *Nat Genet* 2015;47:668-71.
 89. Adam R, Spier I, Zhao B, et al. Exome Sequencing Identifies Biallelic MSH3 Germline Mutations as a Recessive Subtype of Colorectal Adenomatous Polyposis. *Am J Hum Genet* 2016;99:337-51.
 90. Hes FJ, Nielsen M, Bik EC, et al. Somatic APC mosaicism: an underestimated cause of polyposis coli. *Gut* 2008;57:71-6.
 91. Aretz S, Stienen D, Friedrichs N, et al. Somatic APC mosaicism: a frequent cause of familial adenomatous polyposis (FAP). *Hum Mutat* 2007;28:985-92.
 92. Tuohy TM, Burt RW. Somatic mosaicism: a cause for unexplained cases of FAP? *Gut* 2008;57:10-2.
 93. Farrington SM, Dunlop MG. Mosaicism and sporadic familial adenomatous polyposis. *Am J Hum Genet* 1999;64:653-8.
 94. Davidson S, Leshanski L, Rennert G, et al. Maternal mosaicism for a second mutational event--a novel deletion--in a familial adenomatous polyposis family harboring a new germ-line mutation in the alternatively spliced-exon 9 region of APC. *Hum Mutat* 2002;19:83-4.
 95. Baert-Desurmont S, Piton N, Bou J, et al. A remarkable APC mosaicism with two mutant alleles in a family with familial adenomatous polyposis. *Am J Med Genet A* 2011;155A:1500-2.
 96. Jones AC, Sampson JR, Cheadle JP. Low level mosaicism detectable by DHPLC but not by direct sequencing. *Hum Mutat* 2001;17:233-4.
 97. Necker J, Kovac M, Attenhofer M, et al. Detection of APC germ line mosaicism in patients with de novo familial adenomatous polyposis: a plea for the protein truncation test. *J Med Genet* 2011;48:526-9.
 98. Out AA, van Minderhout IJ, van der Stoep N, et al. High-resolution melting (HRM) re-analysis of a polyposis patients cohort reveals previously undetected heterozygous and mosaic APC gene mutations. *Fam Cancer* 2015;14:247-57.
 99. Yamaguchi K, Komura M, Yamaguchi R, et al. Detection of APC mosaicism by next-generation sequencing in an FAP patient. *J Hum Genet* 2015;60:227-31.
 100. Spier I, Drichel D, Kerick M, et al. Low-level APC mutational mosaicism is the underlying cause in a substantial fraction of unexplained colorectal adenomatous polyposis cases. *J Med Genet* 2016;53:172-9.
 101. Jansen AM, Crobach S, Geurts-Giele WR, et al. Distinct Patterns of Somatic Mosaicism in the APC Gene in Neoplasms From Patients With Unexplained Adenomatous Polyposis. *Gastroenterology* 2017;152:546-549 e3.
 102. Goldberg Y, Halpern N, Hubert A, et al. Mutated MCM9 is associated with predisposition to hereditary mixed polyposis and colorectal cancer in addition to primary ovarian failure. *Cancer Genetics* 2015;208:621-624.
 103. Horpaopan S, Spier I, Zink AM, et al. Genome-wide CNV analysis in 221 unrelated patients and targeted high-throughput sequencing reveal novel causative candidate genes for colorectal adenomatous polyposis. *Int J Cancer* 2015;136:E578-89.
 104. Spier I, Kerick M, Drichel D, et al. Exome sequencing identifies potential novel candidate genes in patients with unexplained colorectal adenomatous polyposis. *Familial Cancer* 2016;15:281-288.
 105. Krepischi AC, Achatz MI, Santos EM, et al. Germline DNA copy number variation in familial and early-onset breast cancer. *Breast Cancer Res* 2012;14:R24.
 106. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004;10:789-99.
 107. Traver S, Coulombe P, Peiffer I, et al. MCM9 Is Required for Mammalian DNA Mismatch Repair. *Mol Cell* 2015;59:831-9.
 108. Liu Q, Hesson LB, Nunez AC, et al. Pathogenic germline MCM9 variants are rare in Australian Lynch-like syndrome patients. *Cancer Genet* 2016;209:497-500.
 109. Magalhaes B, Peleteiro B, Lunet N. Dietary patterns and colorectal cancer: systematic review and meta-analysis. *Eur J Cancer Prev* 2012;21:15-23.
 110. Aleksandrova K, Pischon T, Jenab M, et al. Combined impact of healthy lifestyle factors on colorectal cancer: a large European cohort study. *BMC Med* 2014;12:168.
 111. Mehra K, Berkowitz A, Sanft T. Diet, Physical Activity, and Body Weight in Cancer Survivorship. *Med Clin North Am* 2017;101:1151-1165.

112. Kerr J, Anderson C, Lippman SM. Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. *Lancet Oncol* 2017;18:e457-e471.
113. Song M, Garrett WS, Chan AT. Nutrients, Foods, and Colorectal Cancer Prevention. *Gastroenterology* 2015;148:1244-1260.e16.
114. Bradbury KE, Appleby PN, Key TJ. Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Am J Clin Nutr* 2014;100 Suppl 1:394s-8s.
115. Garrett WS. Cancer and the microbiota. *Science* 2015;348:80-6.
116. Mima K, Ogino S, Nakagawa S, et al. The role of intestinal bacteria in the development and progression of gastrointestinal tract neoplasms. *Surgical Oncology* 2017;26:368-376.
117. Dubinkina VB, Tyakht AV, Odintsova VY, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome* 2017;5:141.
118. Dudbridge F, Brown SJ, Ward L, et al. How many cases of disease in a pedigree imply familial disease? *Ann Hum Genet* 2017.
119. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-86.

