Extra-colonic cancers in Lynch Syndrome

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Contents

Abstract

Lynch Syndrome (LS) is the most common of the hereditary colorectal cancer (CRC) syndromes. It is caused by germ-line mutations in one of the four mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* or *PMS2*. Mutations in one of these genes also predispose to several other types of cancer, among these is endometrial cancer the most common. Most estimates on cancer risk associated with MMR mutation have so far been based on retrospective studies. Identification of families with deleterious MMR mutation is of importance, as surveillance may lead to early detection and cure of cancer. The identification of mutation carriers is today mainly based on evaluation of family history of cancers, classification of the families according to the Amsterdam and/or Bethesda clinical criteria and genetic testing of tumour tissue and blood. This work is challenged by the fact that many families with MMR mutations do not fulfill the clinical criteria. The overall aim of the study was to describe extra-colonic cancers occurring in known MMR mutation carriers, and to calculate the sensitivity of the clinical criteria to identify families with deleterious MMR mutation.

By prospectively following women with demonstrated MMR mutation and women belonging to families with aggregation of cancers suggestive of LS but without identified mutation we observed that increased risk of endometrial cancer may be restricted to MMR mutation carriers.

Prostate cancer was not known to be associated with LS. Immunohistochemical analysis (IHC) of tumour tissue from prostate cancers in known MMR mutation carriers demonstrated that the tumours were caused by the mutation. In the series examined prostate cancer occured with a higher frequency, at a younger age and more advanced stage than expected in a similar group of men without known hereditary predisposition to prostate cancer.

The Kaplan-Meier algorithm was used to calculate crude and disease specific survival in female MMR mutation carriers who had contracted ovarian cancer. Eighty-one point five percent of the ovarian cancers were diagnosed as FIGO stage 1 or 2, and 10-year ovarian cancer specific survival independent of staging was 80.6%. This is in contrast to what has been reported for sporadic ovarian cancer and ovarian cancer caused by mutation in *BRCA1* or *BRCA2*, where 10-year survival is less than 50%.

By reclassifying all families who had been demonstrated to have a MMR mutation by November 2009 according to the original and revised Amsterdam and Bethesda clinical criteria we found that less than half of families with *MSH6* mutations would have been identified by the revised Amsterdam criteria.

The combined findings in the studies comprising this thesis emphasize the importance of regarding LS not solely as a CRC syndrome, but as a multi organ cancer syndrome. They also confirm that the clinical criteria in use today to select families for genetic testing will fail to identify a number of mutation carriers and reflect that the different MMR genes may have different expression and penetrance.

List of publications

Paper I

Grindedal EM, Blanco I, Stormorken A, Maehle L, Clark N, González S, Capella G, Vasen H, Burn J, Møller P. High risk of endometrial cancer in colorectal cancer kindred is pathognomonic for MMR-mutation carriers. Fam Cancer. 2009;8(2):145-51. Epub 2008 Oct 8.

Paper II

Grindedal EM, Møller P, Eeles R, Stormorken AT, Bowitz-Lothe IM, Landrø SM, Clark N, Kvåle R, Shanley S, Maehle L. Germ-line mutations in mismatch repair genes associated with prostate cancer. Cancer Epidemiol Biomarkers Prev. 2009 Sep;18(9):2460-7. Epub 2009 Sep 1.

Paper III

Grindedal EM, Renkonen-Sinisalo L, Vasen H, Evans G, Sala P, Blanco I, Gronwald J, Apold J, Eccles DM, Sánchez AA, Sampson J, Järvinen HJ, Bertario L, Crawford GC, Stormorken AT, Maehle L, Moller P. Survival in women with MMR mutations and ovarian cancer: a multicentre study in Lynch syndrome kindreds. J Med Genet. 2010 Feb;47(2):99-102. Epub 2009 Jul 26.

Paper IV

Sjursen W, Haukanes BI, Grindedal EM, Stormorken A, Engebretsen LF, Jonsrud C, Bjørnevoll I, Andresen PA, Adriansen S, Lavik LA, Gilde B, Knappskog P, Fiskerstrand T, Hanslien E, Mæhle L, Møller P. Current clinical criteria for Lynch syndrome are insensitive to identify *MSH6* mutation carriers. Journal of Medical Genetics. In press.

Glossary

Abbreviations

AFAP	Attenuated Familial Adenomatous Polyposis
AMS	Amsterdam criteria
AMSI	Amsterdam criteria I
AMSII	Amsterdam criteria II
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
BI	Bethesda criteria I
BII	Bethesda criteria II
CRC	Colorectal cancer
FAP	Familial Adenomatous Polyposis
IHC	Immunohistochemical Analysis
MSI	Microsatellite instability
MSI-H	High frequency MSI
MSI-L	Low frequency MSI
LS	Lynch Syndrome
MAP	*MUTYH* Associated Polyposis
MMR	Mismatch repair
PSA	Prostate Serum Antigen

Definitions

Allele: One of two or more differing forms of a given gene occupying the same locus on a particular chromosome (Kahl 1995).

Autosome: Any nuclear chromosome other than the sex chromosomes; 22 pairs in the human karyotope. A disease caused by mutation in an autosomal gene or gene pair shows autosomal inheritance (Nussbaum, Mc Innes and WIllard, 2001).

Chromosome: A discrete unit of genome carrying many genes. It consists both of a long DNA-strand and an about an equal mass of proteins (Lewin 2000).

Cumulative risk/Cumulative incidence: Incidence from birth to a given age.

Dominant inheritance: A trait is dominantly inherited if it is phenotypically expressed in heterozygotes (Nussbaum, Mc Innes and WIllard, 2001).

DNA (Deoxyribonucleic acid): A nucleotide polymer that carries the genetic information of viruses, bacteria, and all higher organisms. DNA may occur single- stranded (ssDNA, as in some viral genomes) or double-stranded (dsDNA as in organelles, and chromosomes of all higher organisms). In dsDNA two complementary strands are wound around each other in opposite orientations. The two strands are held together by hodrogen bonds between complementary bases (A=T; G≡C). The sequence of bases in each strand encodes the genetic information (genetic code) (Kahl 1995).

Expression: The appearance of a phenotypic trait as a consequence of the transcription of a specific gene (or specific genes) (Kahl 1995).

Epigenetic: The term that refers to any factor that can affect the phenotype without change in the genotype (Nussbaum, Mc Innes and WIllard, 2001).

Frameshift mutation: A mutation involving a deletion or insertion that is not an exact multiple of three base pairs and thus changes the reading frame of the gene downstream of the mutation (Nussbaum, Mc Innes and WIllard, 2001).

Genotype: The genetic constitution of an individual, either overall or at a specific location (Strachan and Read, 1999).

Germ-line: The germ cells (sperm cells and egg cells) and those cells which give rise to them (Strachan and Read, 1999).

Homologous chromosomes: The two copies of a chromosome in a diploid cell. One copy was inherited from the father and the other from the mother (Strachan and Read, 1999).

Metachronous: Occurring at different times (Dorland's Illunstrated Medical Dictionary, 1974).

Missense mutation: A nucleotide substitution that results in an amino acid change (Strachan and Read, 1999).

Mutation: Any change in the sequence of genomic DNA (Lewin 2000). When using this definition, not all mutations will be pathogenic. In this thesis, *mutation* will be used to denote changes of the DNA that cause an increased risk of disease.

Nonsense mutation: A change in DNA that causes a stop codon to replace a codon that represents an aminoacid (Lewin 2000).

Pathognomonic: Specifically distinctive or characteristic of a disease or pathologic condition; a sign or symptom on which a diagnosis can be made (Dorland's Illunstrated Medical Dictionary, 1974).

Penetrance: The fraction of individuals with a genotype known to cause a disease who have any signs or symptoms of the disease (Nussbaum, Mc Innes and WIllard, 2001).

Phenocopy: A mimic of a phenotype that is usually determined by a specific genotype, produced instead by the interaction of some environmental factor with a normal genotype (Nussbaum, Mc Innes and WIllard, 2001).

Phenotype: The observable structural and functional properties of an organism which results both from its genotype and the environment (Kahl, 1995).

Prevalence: The total number of cases of a disease in existence at a certain time in a designated area (Dorland's Illunstrated Medical Dictionary, 1974).

Proband: The affected person in the family or the person who is seeking genetic advice (Skirton and Patch 2002).

Recessive inheritance: A trait is recessively inherited if it is expressed only in homozygotes or hemizygotes (Nussbaum, Mc Innes and WIllard, 2001).

Recombinant chromosome: A chromosome that results from exchange of reciprocal segments by crossing over between a homologous pair of parental chromosomes during meiosis (Nussbaum, Mc Innes and WIllard, 2001).

Sensitivity (of a diagnostic test): Number with an abnormal test result divided by number of affected.

Specificity (of a diagnostic test): Number with a normal test result divided by number not affected.

Synchronous: Occurring at the same time (Dorland's Illunstrated Medical Dictionary, 1974).

1. General Introduction

1.1 Hereditary colorectal cancer

Between 20 and 25% of all colorectal cancers (CRC) are considered to be familial or hereditary (de la Chapelle, 2004), and between 5 and 10% are caused by mutations in genes that confer a high risk of disease (Abdel-Rahman et al., 2006). The disposition to the syndromes involving a high risk of CRC is mostly inherited in an autosomal dominant fashion (Abdel-Rahman et al., 2006).

The focus of this thesis is Lynch Syndrome (LS). The following syndromes will therefore be described only in brief.

1.1.1 Familial Adenomatous Polyposis (FAP)

FAP accounts for less than 1% of all CRC (Bülow 2003) and is caused by mutations in the *APC* gene. De novo mutations occur in between 22 and 46% of cases. The syndrome is characterized by the development of hundreds to thousands of adenomas in the colon and rectum during childhood and adolescence. These will inevitably develop into cancers around 40 years of age if undetected. Pentrance is almost 100% (Reviewed by Cruz-Correa and Giardiello, 2003).

Patients are at increased risk of developing extra-colonic manifestations. Among these are desmoids, osteomas, congenital hypertrophy of the retinal pigment epithelium (CHRPE) and

dental abnormalities. There is also an increased risk of extra-colonic cancers such as cancer of the thyroid, liver, bile ducts and central nervous system (Reviewed by Half et al., 2009).

A milder form of FAP has been described and denoted attenutated familial adenomatous polyposis (AFAP). AFAP is characterized by fewer than 100 adenomas, a higher age of onset and a lower risk of CRC. Most mutations in *APC* associated with AFAP have been detected in the 5'end or 3'end of the gene, or in exon 9 (Reviewed by Knudsen 2003).

1.1.2 *MUTYH* assosciated polyposis (MAP)

MAP is an autosomal recessive disease caused by biallelic mutations in the *MUTYH* gene, and characterized by a predisposition to multiple adenomas and CRC (Jones et al., 2002). Biallelic *MYH* mutations may account for up to 2.8% of CRC diagnosed before 55 years of age (Fleischmann et al., 2004). In a study by Sampson and colleagues it was reported that mean age of diagnosis was 46 years. Forty eight percent of patients had CRC at time of diagnosis, with a mean age of 50 years (Sampson et al., 2003). Cumulative risk of CRC by 70 years may be as high as 80% (Jenkins et al., 2006). It is not clear whether monoallelic mutation carriers are at increased risk of CRC (reviewed by Kastrinos and Syngal 2007).

1.1.3. Hamartomatous polyposes

These syndromes account for less than 1% of all colorectal cancers. They are characterized by an overgrowth of cells native to the area in which they normally occur, and are also associated with an increased risk of gastrointestinal and extraintestinal cancers. The predisposition to the syndromes is inherited autosomal dominantly, and de novo mutations are common (Reviewed by Schreibman et al., 2005).

Juvenile Polyposis

This is the most common of the hamartomatous syndromes with an incidence of 1 per 100,000 births. Two genes have been identified to cause Juvenile Polyposis, *MADH4* (also known as *SMAD4*) and *BMPR1A*. Mutations in these genes are associated with multiple hamartomatous polyps in the colon and rectum and an increased risk of gastrointestinal cancers (Reviewed by Schreibman et al., 2005).

Cowden syndrome

Prevalence of Cowden syndrome is 1 per 200,000, and it is caused by mutations in the *PTEN* gene. The syndrome is characterized by multiple hamartomatous tumours in the skin, intestine, breast and thyroid gland. There is an increased risk of breast and thyroid cancer (Reviewed by Schreibman et al., 2005).

Peutz-Jegher syndrome

Peutz-Jegher syndrome has a prevalence of 1 in 200,000. The causative gene is *STK11*. The syndrome is associated with gastrointestinal and extraintestinal hamartomatous polyps and mucocutaneous hyperpigmentation. Patients are at increased risk of developing intestinal and extraintestinal cancers (Reviewed by Schreibman et al., 2005).

1.2 Lynch Syndrome

Lynch Syndrome (LS) is the most common of the hereditary colorectal cancer syndromes, and is the focus of this thesis. It is a hereditary multi organ cancer syndrome caused by germline mutations in one of the four genes mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* or

PMS2. The predominant cancers observed in the syndrome are colorectal -and endometrial cancer.

1.2.1 History

The pathologist Aldred Scott Warthin was the first to describe the syndrome. In 1913, he described a family (family "G") with an aggregation of cancers of the colon, endometrium and stomach over several generations. He referred to the syndrome as the "Cancer Family Syndrome" (Warthin AS 1913). Henry T. Lynch published an update of the family including data on more than 650 family members in 1971. He noted that the syndrome seemed to be characterized by an increased risk of early onset adenocarcinomas (especially of the colon and endometrium) increased risk of multiple cancers and an autosomal dominant mode of inheritance (Lynch HT et al., 1971). It was named Lynch Syndrome, and a distinction was made between Lynch syndrome I and Lynch syndrome II, the first referring to families with only colorectal cancers. The second referred to families that also included other forms of cancer, among these, endometrial cancer (Lynch et al., 1985).

To perform studies to identify the genes, and to make a distinction between LS and FAP, LS was renamed Hereditary Non Polyposis Colorectal Cancer (HNPCC) (Vasen et al., 1991). Upon the identification of the three genes *MLH1*, *MSH2*, *MSH6* it became clear that mutations in these could explain some, but not all families with an aggregation of cancer suggestive of HNPCC. The term "HNPCC" was nevertheless used on families with and without detected mutation. Because of this inexact use of "HNPCC" and because this name does not reflect the multi organ cancer nature of the syndrome, LS has become the preferred term for families with a demonstrated germline mutation. Families with aggregation of colorectal cancers without detectable MMR mutation are commonly referred to as familial colorectal cancer (Vasen 2007).

1.2.2 Prevalence

Several studies have been performed to calculate the prevalence of LS providing varying estimates. The variation may be due to differences in the criteria used to define a LS patient, and how many of the four genes one has tested for. When the term LS is used strictly on patients who has tested positive for a MMR mutation, the proportion of all CRC that could be attributed to LS may be between 1 and 3% (Reviewed in de la Chapelle 2005).

1.2.3 Clinical criteria

There are two sets of clinical criteria used for selecting possible LS families for clinical follow-up and genetic testing. These are the original and revised Amsterdam criteria (AMSI and AMSII) and the original and revised Bethesda criteria (BI and BII).

The Amsterdam criteria (AMSI)

The Amsterdam criteria were originally developed for research purposes to identify the genes causing dominantly inherited early onset colorectal cancer (Vasen et al, 1991).

-At least three relatives with histologically verified colorectal cancer, one of which should be a first-degree relative of the other two.

-At least two successive generations should be affected.

-At least one should be diagnosed before 50 years of age.

-Familial adenomatous polyposis should be excluded.

The revised Amsterdam criteria (AMSII)

Following the identification of the genes it became clear that they predisposed to a broad range of cancers, among these were endometrial cancer, cancer of the ureter and renal pelvis and

cancer of the small bowel. The original criteria were therefore revised to include these cancers (Vasen et al 1999).

The Bethesda criteria (BI)

The Bethesda criteria were developed to identify mutation positive families with greater sensitivity, and to select families for testing for microsatellite instability (MSI) of tumour tissue (Rodrigues-Bigas et al., 1997).

1. Individuals with cancer in families that meet the Amsterdam Criteria.

2. Individuals with two HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers, including endometrial, ovarian, gastric, hepatobiliary, or small-bowel cancer or transitional cell carcinoma of the renal pelvis or ureter.

3. Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age <45 y, and the adenoma diagnosed at age <40 y.

4. Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 y.

5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed at age <45.

6. Individuals with signet-ring-cell-type colorectal cancer diagnosed at age <45 y.

7. Individuals with adenomas diagnosed at age <40 y.

Meeting any of the criteria is sufficient.

The revised Bethesda criteria (BII)

The original Bethesda criteria were revised to include any cancer known at the time to be associated with a mutation in one of the MMR-genes, and also patients with microsatellite instable tumours (Umar et al., 2004).

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.

2. Presence of synchronous, metachronous colorectal, or other HNPCC associated tumors, colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumors, sebaceous gland adenomas and keratoacanthomas in Muir–Torre syndrome, and carcinoma of the small bowel regardless of age.

3. Colorectal cancer with the MSI-H histology diagnosed in a patient who is less than 60 years of age.

4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years.

5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

Meeting any of the criteria is sufficient.

Several studies have provided estimates of the sensitivities of the clinical criteria. The estimates may vary according to how many of the MMR genes one has tested for, and whether testing has been performed on unselected colorectal cancer cases or in families fulfilling clinical criteria for HNPCC. The sensitivity of the AMSII criteria to identify mutation carriers may be as low as 40%. The Bethesda criteria have around 90% sensitivity (Reviewed in Vasen et al., 2007).

1.2.4 Molecular genetics

Germline mutations in the MMR genes *MLH1*, *MSH2*, *MSH6* and *PMS2* cause LS. MMR-genes as a group encode proteins that recognize and correct errors that occur during DNA replication. The major function of the DNA mismatch repair system is the elimination of base-base mismatches and insertion-deletion loops. The gene products from *MSH2* and *MSH6* form the hMutSα heterodimer which recognizes base-base mismatches (Drummond et al., 1995). The proteins encoded by *MLH1* and *PMS2* form the hMutLα heterodimer (Li and Modrich 1995). This heterodimer plays the role of a "molecular match maker" and coordinates the mismatch repair system (Reviewed in Jiricny and Nyström-Lahti 2000).

In a review of the international database on mutations identified in the MMR genes from 2004, it was reported that 50% of mutations were detected in *MLH1*, 39% in *MSH2* and 7% in *MSH6* (Peltomäki and Vasen 2004). This may reflect that mutations in *MLH1* and *MSH2* are more prevalent than mutations in *MSH6* in *PMS2*. However, it may also reflect that testing for mutations in *MLH1* and *MSH2* has been performed for a longer time than testing for mutations in the two other genes.

Other MMR genes have been identified; *MLH3*, *MSH3*, *MSH4*, *MSH5* and *PMS1*. It has been suggested that mutations in the *MLH3* gene may be involved in LS (Wu et al., 2001, Liu et al., 2003). However, several other reports have not been able to confirm this possible association (Loukola et al., 2000, Lipkin et al., 2001, Hienonen et al., 2003, Ou et al., 2009). No deleterious mutations have been demonstrated in the four other genes (Reviewed by Peltomäki 2005).

Most of the mutations reported to be associated with LS are frameshift or nonsense mutations that lead to truncated proteins. Missense mutations are also commonly reported in *MLH1* and

MSH6 (Peltomäki and Vasen 2004). Some reports have described inherited epimutations in *MLH1* and *MSH2* (Suter et al., 2004, Chan et al., 2006, Hitchins et al., 2007).

Full DNA analysis of the MMR genes has until now been time consuming and expensive. However, once a pathogenic mutation has been identified, a genetic test for this specific mutation can be offered to the patient's relatives. This test is cheaper and faster, and enables the identification of those at increased risk of cancer who need screening.

1.2.5 Microsatellite instability (MSI)

Repeated DNA sequences called microsatellites can be found throughout the genome. Their repetitive nature makes them susceptible to replication errors. Errors in microsatellites are called microsatellite instability (MSI). Germline mutations in the MMR-genes may inhibit the repair of mismatches occurring during DNA-replication and cause MSI. MSI can be identified in tumour tissue, and is present in most colorectal and endometrial cancers caused by germline mutations in the MMR-genes (de Leeuw et al., 2000, Hendriks et al., 2003, Hendriks et al., 2004). MSI analysis is therefore used in the identification of MMR mutation carriers. To ensure uniform detection of MSI it has been recommended that a panel of five markers is used. Tumours are characterized as displaying high-frequency MSI (MSI-H) if two or more markers show instability and low-frequency MSI (MSI-L) if one of the markers shows instability (Boland et al., 1998). The advantage of MSI analysis is that it may identify patients with germline mutations in yet unidentified genes. However, MSI is also described in a about 15% of sporadic CRC (Ionov et al., 1993) and MSI analysis alone is therefore not a reliable method to identify mutation carriers. MSI in sporadic CRC is almost exclusively due to hypermethylation of the *MLH1* promotor (Kane et al., 1997, Cunningham et al., 1998).

1.2.6 Immunohistochemistry (IHC)

Expression of the MMR-genes in tumour tissue can be detected by immunohistochemical analysis (IHC) using antibodies for the respective proteins. IHC of colorectal tumors in Lynch syndrome families has shown that the gene product from the mutated MMR gene is absent in tumour tissue and at the same time present in adjacent normal tissue (Leach et al., 1996, Thibodeau et al., 1996). IHC has a high sensitivity in the identification of patients with a germ-line MMR-mutation (Hendriks 2003, Hendriks 2004, Halvarsson et al., 2004, Stormorken 2005). The advantage of this method compared to MSI analysis is that it may identify which MMR gene is mutated in the family (Stormorken et al., 2005). An informative result requires that adjacent normal tissue displays presence of gene product from the MMR genes. Thus, each slide is also its own control.

Genetic testing of tumour tissue by MSI-analysis and IHC has mostly been performed on CRC cases, but the methods have also increasingly been performed on selected and unselected endometrial cancer cases (Parc et al., 2000, Berends et al., 2003, Vasen et al., 2004, Hampel et al., 2006, Lu et al., 2007, Walsh et al., 2010), ovarian cancer cases (Jensen et al., 2008), and synchronous endometrial- and ovarian cancers (Soliman et al., 2005, Walsh et al., 2010).

A useful aspect of IHC and MSI analysis is that these methods makes it possible to investigate whether a tumour arising in a MMR mutation carrier is caused by the mutation or whether it is a phenocopy. This has been demonstrated in several case reports (Soravia et al., 2003, Broaddus et al., 2004, Stulp et al., 2008, Yu et al., 2009).

1.2.7 Tumorigenesis

Mutations in MMR genes causing loss of MMR function may contribute to tumorigenesis in several ways. Because of their role in DNA repair, it was originally hypothesized that loss of

MMR function contributed to tumorigenesis because it created a cell that accumulated more and more mutations at an increased rate. Loss of MMR function did not directly cause tumorigenesis, but increased the likelihood of mutations occurring in other proto-oncogenes and tumor suppressor genes. Mutations in the coding regions of several genes have been demonstrated in tumours displaying micorsatellite instability. However, it is not clear whether these acquired mutations actually contribute to tumorigenesis (Reviewed by Heinen 2009).

The MMR genes also play a role in the activation of cell cycle checkpoints and apoptosis when a cell is exposed to certain DNA-damaging agents. Accordingly, it has been proposed that loss of MMR function contributes to tumorigenesis because apoptosis is not activated, and the cell survives even though its DNA is damaged (Reviewed by Heinen 2009).

In addition to being involved in the repair of mismatched base pairs that occur during DNA replication, the MMR genes are also involved in recombination of homologous DNA. This function may play a role in tumorigenesis. Loss of MMR function may inhibit the correction of replication errors occurring during recombination and may also cause chromosomal rearrangements (Wijnen, 1999).

1.2.8 Clinical features

LS is a multi organ cancer syndrome where the predominant phenotypes are colorectal cancer and endometrial cancer. Mutation carriers have up to 80% lifetime risk of colorectal cancer and up to 60% lifetime risk of endometrial cancer. Colorectal cancer risk may be higher in men than in women (Aarnio M et al, 1995, Aarnio M et al., 1999, Jenkins et al., 2006, Choi et al., 2009, Stoffel et al., 2009). There is also an increased risk of metachronous disease (Aarnio et al., 1995).

A common characteristic of hereditary cancer syndromes is early onset of disease compared to its sporadic counterpart. Mean age of CRC onset is considered to be about 45 years (Lynch and de la Chapelle 1999). However, in a recent study where genetic testing was performed on unselected CRC cases, only 44% of patients that proved to be MMR mutation carriers were younger than 50 years and mean age of onset was 50.1 years (Hampel et al., 2008).

Cancer risk according to gene

Cancer risks may vary according to which gene is mutated. Mutations in *MLH1* may confer a higher CRC risk than mutations in *MSH2* and *MSH6* (Stoffel et al., 2009). *MSH2* mutations may confer a higher risk of extra- colonic cancers than *MLH1* mutations (Lin et al., 1998, Vasen et al., 2001). Mutations in *MSH6* have often been reported to be associated with an atypical form of HNPCC (Akiyama et al., 1997, Kolodner et al., 1999, Wagner et al., 2001), and may provide a lower risk of CRC and a later age of onset of CRC (Plaschke et al., 2004), but a higher risk of endometrial cancer than mutations in *MLH1* and *MSH2 (*Ramsoekh et al., 2009). Little is so far known about the expression of *PMS2* mutations, but a recent report proposes that it confers a lower cancer risk than the other MMR genes (Senter et al., 2008). It may be important to note that most reports on cancer risk in HNPCC and/or MMR mutation carriers have so far been based on retrospective data. This may have influended the observations.

Extra-colonic cancer risk

Germ-line MMR mutation is also associated with increased risk of other cancer types, but the risk estimates vary greatly. There may be up to 13% lifetime risk of ovarian cancer, gastric cancer and cancer of the urinary tract respectively, up to 7% lifetime risk of small-bowel cancer, 4% lifetime risk of brain tumours and 2% lifetime risk of hepatobiliary cancers (Reviewed in Vasen et al., 2007). Of the extra-colonic cancers known to be associated with a mutation in the MMR-genes, only endometrial cancer, cancer of the urinary tract and cancer of the small bowel are included in the revised Amsterdam Criteria (Vasen et al., 1999).

1.2.9 Other syndromes associated with mutations in the MMR genes

Muir-Torre syndrome

Muir-Torre syndrome is a phenotypic variant of LS. It is characterized by multiple sebaceous neoplasms and keratoacanthomas and is associated with internal malignancy (Reviewed in Ponti and Ponz de Leon 2005).

Turcot syndrome

In this syndrome there is a co-occurrence of multiple colorectal neoplasms (adenomas or carcinomas) and tumours of the central nervous system (Hamilton et al., 1995). It may be inherited in an autosomal recessive fashion and be caused by two mutations in the same MMR gene (Miyaki et al., 1997, De Rosa et al., 2000).

Neurofibromatosis type 1

Children with bi-allelic mutations in one of the MMR genes may develop café au lait spots and early onset malignancies, which are clinical signs of Neurofibromatosis type 1. The cancers that occur in bi-allelic mutation carriers are brain tumours, haematological malignancies and CRC. Brain tumours and haematological malignancies often occur in the first decade of life, and CRC in the second (Reviewed in Bandipalliam 2005).

1.2.10 Surveillance

MMR mutation carriers are offered surveillance according to the Mallorca guidelines (Vasen et al., 2007). This surveillance includes biannual colonoscopies from the age of 25 and onwards and biannual screening of the endometrium with transvaginal ultrasound. The aim of the surveillance is prevention and early detection of cancers through removal of polyps before they become cancerous.

Colonoscopic surveillance every 3rd year reduces CRC risk with more than 50% (Järvinen et al., 2000), leads to early detection of CRC, and reduces CRC mortality (Renkonen-Sinisalo et al., 2000, de Jong et al., 2006). However, interval cancers have been reported in patients undergoing colonoscopies every third year (Renkonen-Sinisalo et al., 2000). The Mallorca group has therefore recommended that the interval should be between 1 and 2 years (Vasen et al., 2007).

Endometrial screening with ultrasound may lead to early detection of endometrial cancer, but the effect of such screening has not been clarified (Dove-Edwin et al., 2002, Rijken et al., 2003).

Screening for early detection of cancer of the urinary tract and gastric cancer has been offered in selected families.

Prophylactic oophorectomy reduces risk of ovarian cancer in female MMR mutation carriers (Schmeler et al., 2006) and it has been discussed whether this should be considered after child bearing (Bertagnolli 2005, Lu 2008, Schmeler 2008).

1.2.11 Survival

It has been reported that CRC in HNPCC families is diagnosed at an earlier stage and with fewer distant metastasis than sporadic CRC (Watson et al., 1998). CRC survival matched for stage at diagnosis may also be better in HNPCC-related CRC than in sporadic CRC (Watson et al., 1998, Stigliano et al., 2008). However, conflicting reports exist (Bertario et al., 1999,

Barnetson et al., 2006), and an explanation for the possible survival advantage remains unknown.

Endometrial cancer and ovarian cancer in known MMR mutation carriers or in families fulfilling the AMSII criteria are reported to have similar survival rates as sporadic endometrial and ovarian cancer when matched for stage and age (Boks et al., 2002, Crijnen et al., 2005). However, knowledge about survival of the extra-colonic cancers caused by MMR mutations is limited.

1.2.12 Identification of MMR mutation carriers

Because colorectal screening reduces CRC morbidity and mortality, identification of MMR mutation carriers is of great importance. Detection of a MMR mutation will also enable the identification of those who have not inherited an increased risk of cancer. Cancers caused by germ-line MMR mutations have no known pathognomonic signs that facilitate the diagnosis of LS. Identification of LS famililies in Norway is therefore today mainly based on family history. Upon referral, all relevant diagnoses in the family are confirmed from medical files or the Cancer Registry of Norway, and the family is classified according to the Amsterdam and/or Bethesda clinical criteria. Genetic testing by IHC and/or MSI analysis is performed on tumour tissue from one or preferably two affected relatives. In families where one or more of the tumours are MSI-H and/or there is absence of gene product from one or more of the MMR genes, a blood sample from an affected family member is investigated for a germ-line mutation in the gene indicated by IHC to be mutated. If no blood sample is available from an affected member of the family, genetic testing is offered to unaffected close relatives. When a mutation is detected, predictive genetic testing is offered to the extended family. Mutation positive family members are offered screening for prevention and early diagnosis of cancer as described.

1.2.13 Familial colorectal cancer/Familial Colorectal Cancer Type X

Between 40 and 80% of families fulfilling the AMSI criteria, and between 5 and 50% of families fulfilling the AMSII criteria do not have an identifiable MMR mutation (Reviewed in Lynch and de la Chapelle 2003). These families are termed Familial Colorectal Cancer (Vasen et al., 2007) or Familal Colorectal Cancer Type X (Lindor et al., 2005). Lindor and colleagues observed a lower CRC risk and a later age of onset in AMSI families without MMR mutation than in families with mutation (Lindor et al., 2005). Similarly, annual incidence of tubular adenomas is similar in families with and without demonstrable MMR mutation, but the adenomas progress more rapidly to severe dysplasia or cancer in mutation carriers (Stormorken et al., 2007).

2. Aims of the study

Today's estimates on cancer risk associated with MMR mutations are mostly based on retrospective observations and may be influenced by the criteria used for selecting the families. (Early onset) CRC is often central to these. However, not all families with MMR mutation fulfil the clinical criteria, and may therefore not be identified. The cancer risk in these unidentified families is unknown. Knowledge about and attention to the extra-colonic cancers associated with MMR mutation could contribute to the identification of mutation carriers.

The overall aim of the study was to describe extra-colonic cancers occurring in known MMR mutation carriers, and to calculate the sensitivity of the clinical criteria to identify families with deleterious MMR mutation.

The aims of the substudies were:

To describe risk of endometrial cancer in families with and without identifiable MMR mutation

Previous studies on endometrial cancer risk have mainly been based on retrospective data. We wanted to compare prospectively observed annual incidence rates of endometrial cancer in female carriers of MMR mutation and women in families fulfilling the AMSII criteria where no mutation had been detected (Paper I).

To investigate whether MMR germ-line mutation is associated with prostate cancer

Prostate cancer has not been considered part of LS. We wanted to use IHC to investigate whether prostate cancers occurring in MMR mutation carriers were caused by the germ-line mutation. We also wanted to analyse the possible association by comparing observed incidence

and cumulative risk of prostate cancer in known carriers of MMR mutation with expected based on population data (Paper II).

To calculate ovarian cancer survival in MMR mutation carriers

There is limited knowledge about survival from ovarian cancer caused by MMR mutations. The aim of this study was to use the Kaplan-Meier algorithm to calculate ovarian cancer specific survival in women with a deleterious MMR mutation (Paper III).

To calculate sensitivity of clinical criteria

We wanted to reclassify all families with a demonstrated MMR mutation according to AMSI/II and BI/II criteria to calculate sensitivities of the respective criteria to identify mutation positive families (Paper IV).

3. Material and methods

3.1 Patients

Papers I, III and IV were multi-centre studies. Paper I included patients from The Regional Cancer Genetic Service at the Catalan Institute of Oncology, Hospital Duran i Reynalds in Barcelona, Spain, in addition to patients from the department of hereditary cancer at Radiumhospitalet, Oslo University Hospital. Paper III included women with MMR mutation who had been diagnosed with ovarian cancer from altogether 11 European centres for hereditary cancer. Paper IV was a national study involving all Norwegian departments for hereditary cancer and includes all Norwegian families who by November 2009 had been shown to carry MMR mutations.

All papers include patients that belong to families that have been referred to the department of hereditary cancer at Radiumhospitalet, Oslo University Hospital. Once referred, all relevant diagnoses in the families were confirmed from medical records and/or the Cancer Registry of Norway, and the families were classified according to the clinical criteria for hereditary cancer syndromes. Upon classification, genetic testing was performed in the clinical setting with IHC and/or MSI analysis of one or preferably two relatives affected by colorectal cancer and/or other cancers included in the LS tumor spectrum. When IHC and/or MSI-analysis showed abnormal results, genetic testing of blood from an affected in the family was performed. If no blood sample from an affected was available, we tested blood from first degree relatives. Patients with LS (detected MMR mutation) and patients in families with familial colorectal cancer (no MMR mutation identified in family) were offered surveillance according to the Mallorca guidelines (Vasen et al., 2007).

Paper I includes both women who have been identified to be mutation carriers and women belonging to families fulfilling the AMSII criteria but without demonstrated MMR mutation. Papers II, III and IV include only patients from families with identified MMR mutation.

Population data on incidence of cancer was taken from the Cancer Registry of Norway.

3.2 Methods

3.2.1 Immunohistochemistry

In paper II, prostate cancer tumour tissue from men with MMR mutation was analysed with IHC for presence or absence of gene product from *MLH1*, *MSH2*, *MSH6* and *PMS2*. Complete absence of gene product from the mutated gene in tumour tissue was considered to indicate that the prostate cancer was in fact caused by the germ-line mutation. The analyses were done in one laboratory (Department of Pathology, Ullevål University Hospital) and the method has been described in detail previously (Stormorken et al., 2005). The pathologists doing the analysis were blinded for the mutation in the family, and the slides were double read by two pathologists.

3.2.2 Statistics

Comparison of observed and estimated incidence of endometrial and prostate cancer

Data on observed incidence of endometrial cancer (Paper I) and prostate cancer (Paper II) were compared with expected based on population based data from the Cancer Registry of Norway. Observed and expected number of cases was compared with χ^2 statistics.

In paper I we calculated expected number of endometrial cancers to occur during the follow-up years by using age-specific annual incidence rates for age at first control and age at last control. Both rates were multiplied with follow-up years for each patient, and the numbers of cancer expected was estimated to be the mean of the two.

In paper II we calculated the expected number of prostate cancers to occur in a group of men, similar to the men in the study group according to age, but without known hereditary risk of prostate cancer. This was done by using population data on incidence of prostate cancer according to birth cohort, age and observation year. Expected incidence for each lived year under observation was calculated for each man. The expected incidence was then summarized for each man, and then for all men.

Comparison of observed and estimated mean age of onset of prostate cancer

Expected mean age of onset of prostate cancer was derived from population data on incidence according to birth cohort, age, and observation year. Expected number of cancers to occur at all ages (from 0 to 85) was calculated for all men in the study group. For each 1-y age stratum, the number of expected cancers was summarized. The mean age of onset was derived with the following formula:

$$\text{Mean age} = \sum_{i=1}^{85} n_i \cdot age_i \; / \sum_{i=1}^{85} n_i$$

Where “I” designates the 1-y age stratum ranging from 1 to 85, “n” is the number of expected cancers in each stratum, and “age” is the age in years for each stratum. Observed and expected mean age of onset was compared using a one-sample *t* test.

Gleason score

Population data on Gleason score for prostate cancers diagnosed in men younger than 70 years were obtained from the Cancer Registry of Norway. Observed and expected number of cancers with Gleason score <8 and between 8 and 10 were compared with Fisher's exact *P*.

Survival analysis

In paper I, the Kaplan-Meier algorithm was used to visualize annual incidence rates of invasive endometrial cancer in MMR mutation carriers and in women belonging to families meeting the AMSII criteria but where no MMR mutation had been identified. Starting date was date of first follow-up. All women were scored as either affected with endometrial cancer at time of diagnosis or unaffected at last day of follow-up.

In paper II, we used the Kaplan-Meier algorithm to calculate cumulative risk of prostate cancer. The men in the study group were scored as affected at time of prostate cancer diagnosis or unaffected at last date of observation or at death if dead for another reason.

This algorithm was also used in paper III to calculate crude survival, survival from LS related tumours and ovarian cancer specific survival. Starting date was date of ovarian cancer diagnosis, and observation time was censored at last follow-up. Events were scored as death caused by ovarian cancer, death caused by other LS associated tumour, death caused by other cancer, or other cause of death. When calculating survival from LS related tumours, deaths from other cancers and other causes of death were scored as no event. When calculating ovarian cancer specific survival, all other deaths were scored as no event.

3.2.3 Classification of DNA variants

For paper IV, all DNA variants detected in Norwegian families in the four MMR genes were checked against published mutations in the following websites: http://www.insight-group.org (LOVD: Leiden Open Variation Database), https://portal.biobase-international.com/hgmd/pro/start.php (Human Gene Mutation Database), Pub Med and http://www.med.mun.ca/MMRvariants (Woods et al., 2007). Mutations causing direct stop/nonsense, frameshifts, splice defects and large insertions/deletions were considered pathogenic. Missense mutations or small in-frame deletions were subjected to segregation analyses when possible (Stormorken et al., 2003). If a review of the international databases or segregation analyses strongly suggested the variant to be pathogenic, the mutations were scored accordingly. All other DNA variants were considered part of normal variation or the information available of the variant and the family was insufficient for conclusive scoring. These variants were excluded from the report.

3.2.4 Clinical classification

For paper IV, all Norwegian families in which a MMR mutation had been detected were reclassified according to clinical criteria AMSI, AMSII or BII with the information obtained as of November 2009.

3.2.5 Ethics

All activities that form the basis for the papers have been part of the public health care system, and no separate medical files including patient names have been erected. All diagnoses were confirmed with written consent from the patient himself/herself if alive or from descendants if deceased. Written informed consent to all genetic testing of blood or tumour tissue was obtained. If the patient was dead, descendants or other close relatives gave their consent. Series from abroad were constructed according to national legislation.

4. Summary of papers

The present thesis is based on the following papers:

I. High risk of endometrial cancer in colorectal cancer kindred is pathognomonic for MMR mutation carriers.

Eli Marie Grindedal, Ignacio Blanco, Astrid Stormorken, Lovise Mæhle, Neal Clark, Sara González, Gabriel Capella, Hans Vasen, John Burn and Pål Møller

Fam Cancer. 2009;8(2):145-51. Epub 2008 Oct 8.

We performed a prospective study of annual incidence rates of endometrial cancer in women with a known mutation in *MLH1*, *MSH2*, *MSH6* and *PMS2* (Mut+) or who belonged to a family that fulfilled the AMSII criteria but where no mutation had been detected (Ams+). Eighty and 171 women were included in the two respective groups. Annual incidence rates were calculated as events observed after first control/follow-up years. Observed number of cancers was compared with number expected to occur by chance, derived from the Cancer Registry of Norway.

Ten percent of the Mut+ women contracted invasive endometrial cancer compared to only 0.6% in the Ams+ group ($p = 0.0006$). The annual incidence rates in the two groups were 2.5% and 0.2% respectively. Two of the Mut+ women were also diagnosed with a concurrent gynaecological tumour. Observed number of endometrial cancer in the Mut+ group was significantly higher than expected to occur by chance ($p << 0.01$).

Increased risk of endometrial cancer may be restricted to MMR mutation carriers. Our findings support previous reports demonstrating a difference in cancer risk and cancer spectrum between families with a demonstrated MMR mutation (LS) and families were no mutation has been detected (familial colorectal cancer). By the use of prospective data it was confirmed that the combined presence of colorectal and endometrial cancer may be the hallmark of LS.

II. Germ-line Mutations in Mismatch Repair Genes Associated with Prostate Cancer

Eli Marie Grindedal, Pål Møller, Ros Eeles, Astrid Tenden Stormorken, Inger Marie Bowitz-Lothe, Stefan Magnus Landrø, Neal Clark, Rune Kvåle, Susan Shanley and Lovise Mæhle

Cancer Epidemiol Biomarkers Prev. 2009 Sep;18(9):2460-7. Epub 2009 Sep 1.

We investigated whether germ-line MMR mutations may be associated with prostate cancer. One-hundred-and-six male carriers of MMR mutations were identified in our electronic medical files. Within this group, 9 men had been diagnosed with prostate cancer. IHC was performed on tumour tissue from the prostate cancers. Observed incidence, cumulative risk, age of onset and Gleason score were compared with expected based on population data on cancer risk.

In 7 out of 8 available tumours we observed no staining of the gene product from the gene mutated in the patient. Prostate cancer in MMR mutation carriers occurred with a higher incidence ($p < 0.01$), at a lower age ($p = 0.006$) and had a higher Gleason score ($p < 0.00001$) than expected in a similar group of men with no known hereditary risk of prostate cancer.

Cumulative risk by 70 years calculated by Kaplan Meier analysis was 30%, compared to 8% in the general population.

Our observations suggest that the MMR genes may be among the few genes causing a high risk of prostate cancer when mutated. Prospective observations are warranted, but the potential risk of prostate cancer in MMR mutation carriers should be taken into clinical consideration.

III. Survival in women with MMR mutations and ovarian cancer; a multicentre study in Lynch Syndrome kindreds

Eli Marie Grindedal, Laura Renkonen-Sinisalo, Hans Vasen, Gareth Evans, Paola Sala P, Ignacio Blanco, Jacek Gronwald, Jaran Apold, Diana M. Eccles, Ángel Alonso Sánchez, Julian Sampson, Heikki J. Järvinen, Lucio Bertario, Gillian C. Crawford, Astrid Tenden Stormorken, Lovise Maehle and Pål Møller

J Med Genet. 2010 Feb;47(2):99-102. Epub 2009 Jul 26.

Retrospective data on women with a germ-line MMR mutation who had been diagnosed with ovarian cancer was collected from 11 European centres for hereditary cancer. The Kaplan Meier algorithm was used to calculate crude and disease specific survival.

In the series examined, 81.5% of the ovarian cancers were diagnosed as FIGO stage 1 or 2. Ten-year ovarian cancer specific survival independent of staging was 80.6%, compared to less than 50% which has been reported for ovarian cancer caused by mutations in *BRCA1*/*BRCA2*

and sporadic ovarian cancer. The difference in survival could not be contributed to an absence of serous cancers in MMR mutation carriers. Twenty-two point nine percent of the women had a concurrent gynaecological tumour diagnosed with their ovarian cancer and 50% developed a later LS related tumour.

Female MMR mutation carriers may have a lifetime risk of dying of ovarian cancer around 2%. Ovarian cancer caused by germ-line MMR mutations may be biologically different than ovarian cancer caused by mutations in the *BRCA1/2* genes.

IV. Current clinical criteria for Lynch syndrome are not sensitive enough to identify *MSH6* mutation carriers

Wenche Sjursen, Bjørn Ivar Haukanes, Eli Marie Grindedal, Astrid Stormorken, Lars F. Engebretsen, Christoffer Jonsrud, Inga Bjørnevoll, Per Arne Andresen, Sarah Adriansen, Liss Anne Lavik, Bodil Gilde, Per Knappskog, Torunn Fiskerstrand, Eldbjørg Hanslien, Lovise Mæhle and Pål Møller

Journal of Medical Genetics. In press.

We performed a national study including families from all cancer genetics clinics in Norway. Families demonstrated to have a MMR mutation up until November 2009 were reclassified according to the AMSI/II and BII clinical criteria, and sensitivities of the different criteria to identify the mutation carrying families were calculated.

Sixty-nine distinct deleterious MMR mutations had been identified in a total of 129 families. Forty-five percent of mutations were detected in *MSH2*, 27% in *MSH6*, 22% in *MLH1* and 6% in *PMS2*. Thirty-eight percent of *MSH2* families, 12% of *MSH6* families, 78% of *MLH1* families and 25 % of *PMS2* families met the AMSI criteria. Corresponding sensitivity for the AMSII criteria to identify mutations in the different genes were 62%, 48%, 87% and 38%. Similarly, each of the clinical Bethesda criteria had low sensitivity to identify *MSH6* and *PMS2* mutations.

AMSI/II and BI had a low sensitivity in detecting families with a mutation in *MSH6*. Penetrance and prevalence of *MSH6* mutations may be different from current estimates based on fulfilment of the AMSI/II criteria. Our findings suggest that all incident cancers included in the LS tumour spectrum may be subjected to IHC/MSI analysis to increase detection of families with deleterious MMR mutation.

5. General discussion

5.1 Methodological considerations

5.1.1 Study design

The results of retrospective studies should be handled with caution as the observations may be influenced by the selection criteria used to identify the patients.

We performed a retrospective study to investigate whether MMR germ-line mutations could be associated with prostate cancer (Paper II). Prostate cancer is not included in the Amsterdam or Bethesda clinical criteria and has not been considered part of LS. Nevertheless, we cannot exclude that prostate cancer may have been part of the reason for the referral and ascertainment of the family, and that this may have contributed to an overestimation of prostate cancer risk in MMR mutation carriers. Prospective studies of the association of prostate cancer and MMR germ-line mutations are needed to confirm our observations.

Our study of ovarian cancer survival in MMR mutation carriers was also based on retrospective data (Paper III). The women were not selected based on specific characteristics of their ovarian cancers, and the retrospective nature of the data may therefore not have affected the survival estimates. However, the diagnoses had been made over a period of 60 years and in 11 different countries. This limited our possibilities to analyse the histology of the cancers in detail. Moreover, IHC was not performed on all tumours to investigate whether the cancer was caused by the MMR mutation, and we cannot exclude that some of the cancers were sporadic.

5.1.2 PSA-testing

Three of the prostate cancer diagnoses were made on asymptomatic men who had had a PSA test performed regularly (Paper II). We had not advised men in LS families to attend prostate cancer screening. However, we do not know whether these diagnoses would have been made had they not attended screening. This may have caused an overestimation of risk in mutation carriers. The incidence of prostate cancer in Norway increased with 3.9% per year between 1988 and 1999, mainly because of increased use of routine PSA-testing on asymptomatic men (Kvåle et al., 2007). When calculating the expected number of cancers to occur in the study group, we used population data from The Cancer Registry of Norway on incidence at a specific age in a specific year. The increased use of PSA testing in the general population was therefore accounted for, and this may have reduced the possible overestimation of prostate cancer incidence in mutation carriers.

5.2 Discussion of findings

5.2.1 Lynch syndrome is a multi organ cancer syndrome

Increased risk of endometrial cancer may be restricted to MMR mutation carriers (Paper I), prostate cancer may be part of LS (Paper II) and ovarian cancer caused by MMR mutation may be biologically different from sporadic ovarian cancer and ovarian cancer caused by BRCA-mutations (Paper III).

Other studies have demonstrated that the combined presence of endometrial -and colorectal cancer is the main finding to predict MMR mutation in an HNPCC- familiy (Wijnen et al., 1998) and that endometrial cancer risk in particular (Renkonen et al., 2003, Boilesen et al., 2008) and extra-colonic cancer risk in general is higher in families with indication of or demonstrated MMR mutation than in families without (Lindor et al., 2005).

Our findings are in keeping with the extensive literature demonstrating that LS is a multi organ cancer syndrome, and validated that increased risk of extra-colonic cancers may be what distinguishes LS families from families fulfilling Amsterdam and Bethesda clinical criteria where no MMR mutation is identified.

5.2.2 Expression of MMR mutations may change as more cancers are prevented and cured

Germ-line MMR mutation may be associated with prostate cancer and the MMR genes may be among the genes that cause a high risk of prostate cancer when mutated (Paper II). Prostate cancer is not included in the clinical criteria used to identify possible LS families, and has historically not been considered a LS cancer.

Goecke and colleagues (Goecke et al., 2006) reported that in carriers of *MSH2* mutation, prostate cancer was the most common cancer type not included in the LS tumour spectrum. Prostate cancer has also been reported among MMR mutation carriers in other recent reports, but estimates on risk were either not performed (Järvinen et al., 2009, Barrow et al., 2009), or increased risk was not demonstrated (Baglietto et al., 2010). A recent case report presents a family with a probable deleterious missense mutation in *MLH1*, where two male mutation carriers contracted prostate cancer at 50 and 55 years of age (Yu et al 2009). IHC was not performed on tumour tissue from the prostate cancers in any of these studies.

There have been several case reports of cancers traditionally not considered part of LS in MMR mutation carriers that display MSI and/or absence of gene product from the mutated MMR gene. Among these are:

Breast cancer (De Leeuw et al., 2003, Westenend PJ et al., 2005, Blokhuis et al., 2008, Akoum et al., 2009, Jensen et al., 2009)

Male breast cancer (Yu et al., 2009)

Thyroid cancer (Broaddus et al., 2004, Stulp RP et al., 2008)

Adrenal cortical carcinoma (Broaddus et al., 2004)

Liposarcoma (Hirata et al., 2006)

Leiomyosarcoma (Yu et al., 2009)

Non-Hodgkin's lymphoma (Pineda et al., 2008)

These cancers are currently not known to occur with a higher frequency in MMR mutation carriers than in the general population, and systematic screening for early detection has not been discussed. However, attention to these cancers when considering genetic testing for MMR mutation in families with aggregation of cancers may be of importance as it could contribute to identify patients with increased risk of CRC.

Annual or biannual colonoscopy has been shown to reduce colorectal cancer morbidity and mortality in MMR mutation carriers (Stormorken AT et al., 2007, Stupart DA et al., 2009, Engel C et al., 2010). We observed that as many as 50% of the women diagnosed with ovarian cancer developed a concurrent or later cancer (Paper III). As more mutation carriers survive their cancers, we may see a greater number of late-onset cancers, and the penetrance and expression of mutations in the MMR genes may change.

5.2.3 Biology of tumours caused by MMR germ-line mutation

Eighty percent of ovarian cancers caused by MMR germ-line mutation were diagnosed as FIGO stage I or II and 10-year survival independent of FIGO-staging was as high as 80% (Paper III). This is in contrast to what has been reported for ovarian cancer caused by *BRCA1/2*-mutations and sporadic ovarian cancer, where 2/3 of cancers are diagnosed as stage III/IV and 10-year survival is between 36% and 47% (Rosenthal et al 2006, Evans et al 2008, Cancer Registry of Norway, 2008).

MMR deficient ovarian cancers may be characterized by an overrepresentation of nonserous histologic subtypes (Reviewed by Pal T et al., 2008). We observed that about 77% of the cancers with a histological description were described as non-serous. However, we did not find that these had a significantly better survival than the cancers described as serous.

Ovarian cancer caused by MMR mutations may be biologically different from sporadic ovarian cancer and ovarian cancer caused by mutations in the BRCA-genes. However, we could not assess whether ovarian cancers caused by germ-line MMR mutations are characterized by a favourable prognosis, or whether the cancers had such a good prognosis because they were diagnosed early. This question could preferably be addressed by a prospective study.

Prostate cancer occurring in MMR mutation carriers and displaying absence of gene product from the mutated gene has a higher Gleason score than sporadic prostate cancers (Paper II). More studies are needed to assess whether prostate cancers caused by MMR mutations have distinct features and whether these features may affect survival.

It has recently been reported that endometrial cancer of the lower uterine segment is associated with LS (Westin et al., 2008). Cancer of the lower uterine segment may be hard to distinguish from endocervical carcinomas. Two of eight endometrial cancers diagnosed in MMR mutation

carriers were described as carcinomas of the endocervix, but histological reports were not investigated with this in mind (Paper I).

Knowledge about possible characteristics of tumours associated with LS may facilitate identification of MMR mutation carriers. The combined findings of our studies and other reports may indicate that ovarian cancer, prostate cancer and endometrial cancer caused by MMR germ-line mutations may have certain clinicopathological characteristics, but further studies are warranted on this subject.

5.2.4 Prevalence, expression and penetrance of mutations in *MSH6*

Early onset CRC is currently central to the clinical criteria used to identify possible LS families. We observed that less than half of families with *MSH6* mutations fulfilled the AMSII criteria (Paper IV).

The insensitivity of the clinical criteria to identify families with *MSH6* mutations could be due to late onset CRC in these families (Hendriks et al., 2004, Ramsoekh et al., 2009), but it could also indicate that extra-colonic cancers are common. Accordingly, Plaschke and colleagues reported that families with *MSH6* mutations had a lower frequency of CRC, but a higher frequency of cancers not associated with LS (of these were prostate cancer the most common among men) compared to *MSH2* and *MLH1* families. There was also a higher age of onset for cancer in general in *MSH6* mutation carriers (Plaschke et al., 2004).

Twenty seven percent of the MMR mutations identified in Norwegian families were detected in *MSH6* (Paper IV). This is in contrast to what has been observed in a few recent studies where mutations in *MLH1* and *MSH2* have been reported with a higher frequency than mutations in *MSH6* and *PMS2 (*Hampel et al., 2005, Mueller et al., 2009, Berginc et al., 2009).

In 2004, only 7% of the mutations reported to the mutation database run by the InSight group were found in *MSH6*, compared to 50% for *MLH1* and 39% for *MSH2* (Peltomaki et al., 2004). Mutations in *MSH6* may be less common than mutations in *MLH1* and *MSH2*. However, the reported low prevalence may also reflect that testing for mutations in *MLH1* and *MSH2* has been performed for a longer time than testing for mutations in the two other genes. One may also hypothesize that it may be related to our observation that many of these families do not fulfil the criteria used to select patients for genetic testing (Paper IV).

In sum, our observations are in keeping with other studies reporting that the four MMR genes may have different expression and penetrance. Genetic testing on families with aggregation of cancers that are not necessarily typical of LS may increase detection of *MSH6* mutation carriers.

5.2.5 IHC and/or MSI analysis of extra-colonic cancers in LS

Genetic testing of tumour tissue is often performed only in the initial process of identifying a mutation in a family and is commonly done on tumour tissue from colorectal cancers, both unselected and selected cases (Hampel et al., 2005, Lagerstedt Robinson et al., 2007, Julié et al., 2008). IHC and/or MSI analysis of on unselected and selected endometrial cancers have recently been used in the detection of MMR mutation carriers (Hampel et al., 2006, Lu et al., 2007, Garg et al., 2009), and it was demonstrated that most of the women whose tumours showed abnormal IHC and/or MSI or who proved to be mutation carriers did not meet clinical criteria for LS (Hampel et al.2006, Garg et al., 2009).

To improve identification of mutation carriers, the Mallorca Group has recommended IHC/MSI analysis to be performed on all incident colorectal cancer cases (Vasen et al., 2009). IHC/MSI analysis of incident endometrial cancer cases has also recently been advocated (Resnick et al., 2009).

The combined findings of our studies reflect that attention to extra-colonic cancers may be important in the identification of MMR mutation carriers, and that IHC/MSI analysis may be used to identify whether cancers that MMR mutation carriers contract are caused by the mutation or whether they are phenocopies (Papers I-IV). A broad view on what families could be subjected to IHC and/or MSI analysis in cancer genetics clinics, and performance of such analyses of all incident cancers included in the LS tumour spectrum (including prostate cancer) may contribute to the identification of families with MMR mutations. Moreover, systematic performance of IHC and/or MSI analysis of the extra-colonic cancers that MMR carriers contract prospectively could be used to investigate whether they are caused by the germ-line mutation. This may be a valuable tool to precisely describe expression, penetrance and prevalence of mutations in the different MMR genes, and to monitor whether these are changing.

5.2.6 Cancer risk in families fulfilling the AMSII criteria without identifiable MMR mutation

We could not prospectively demonstrate an increased risk of endometrial cancer in women belonging to families fulfilling AMSII criteria without identifiable MMR mutation. Numbers were too low to draw conclusions, but our findings indicated that increased risk of endometrial cancer may be restricted to MMR mutation carriers (Paper I).

Our findings are in keeping with previous retrospective observations. Lindor and colleagues observed no increased risk of extra colonic cancers in families fulfilling Amsterdam criteria without indication of MMR mutation (Lindor et al., 2005). Several studies have also reported a slower progression of adenomas to CRC (Mueller-Koch et al., 2005, Stormorken et al., 2007) and lower CRC risk in these families compared to families with detected MMR mutation (Lindor et al., 2005, Engel et al., 2010).

If increased risk of extra-colonic cancers is restricted to MMR mutation carriers, and all MMR genes predispose to extra-colonic cancers, what remains is families with aggregation of CRC without evidence of MMR mutation by IHC and/or MIS analysis. Lynch denoted families with only CRC Lynch syndrome I (Lynch 1985). It has also been referred to as Familal Colorectal Cancer Type X (Lindor et al., 2005) or familial colorectal cancer (Vasen et al., 2007). It has been hypothesized that it is a heterogenous group. Some aggregation of CRC may be due to chance, some may be due to shared environmental factors, and some may be due to mutations in yet unidentified genes (Lindor et al., 2005), or a combination of these.

6. Conclusions

The combined findings of the studies constituting this thesis emphasize that LS is a multi organ cancer syndrome. They also confirm that the clinical criteria in use today to select families for genetic testing will fail to identify a number of mutation carriers, especially carriers of *MSH6* mutations. This reflects that penetrances and expressions may be different for mutations in the different genes.

Most studies on penetrance and expression of mutations in the MMR genes have so far been based on retrospective data. The insensitivity of the clinical criteria to identify MMR mutations indicate that prospective studies of known mutation carriers are needed for a precise calculation of the risk- and survival of the extra colonic cancers associated with mutations in the four MMR genes, and for an analysis of their clinicopathological traits. To obtain large enough series, such studies may need to be performed as collaborations between several centres for hereditary cancer.

To improve detection of MMR mutation carriers The Mallorca Group has recommended IHC/MSI- analysis to be performed on all incident colorectal cancers (Vasen et al., 2009). Such testing of all incident extra-colonic cancers included in the LS tumour spectrum, including prostate cancer, may increase identification of mutation carriers. In the cancer genetics clinics, a broad view on what families that could be subjected to genetic testing for MMR mutations in a cancer genetics clinic may also lead to increased identification. If more mutation carriers are identified, more precise estimates of prevalence of mutations in the different genes may be made.

IHC/MSI analysis of all extra-colonic cancers that MMR mutation carriers contract prospectively to investigate whether these are caused by the mutation may provide information on the expression and penetrance of mutations in the different genes. As more cancers are

prevented and cured, this information will in turn be valuable to monitor whether the LS tumour spectrum is changing: Persons who previously may have died, may now survive and contract another cancer later.

Several questions considering LS are still unanswered, and may best be answered by prospective studies. The association between prostate cancer and MMR mutation should be validated and prostate cancer specific survival in such patients should be described. Also, prospective studies are warranted to assess whether ovarian cancer survival could be further improved through modern diagnostics and treatment. Such studies could also enable closer histological analysis of these cancers. As so many of the *MSH6* mutation families are not identified by the clinical criteria, the expression and penetrance of mutations in this gene needs further clarification.

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