## THESIS SUMMARY

Lynch syndrome (LS) is a hereditary cancer predisposition to early onset colorectal and endometrium cancers (1, 2). The underlying cause of LS is an inactivating germ-line mutation in one of the DNA mismatch repair (MMR) genes: *MLH1, MSH2, MSH6* or *PMS2* (3–6). Patients are born heterozygous for the germ-line mutation and develop neoplasms upon somatic loss of the one wild-type copy of the gene (7). In the absence of a functional DNA MMR system, cells cannot detect and correct mismatches that may arise in the genome due to erroneous DNA replication or exposure to DNA damaging agents. Consequently, a mutator phenotype will develop that increases the chances of mutations arising in oncogenes and tumor suppressor genes, and hence the development of malignancies (8).

The diagnosis of LS is essential to offer patients the optimal treatment. To date the diagnosis of LS relies on the clinical phenotype, tumor pathology data and genetic screening results. Typical LS cases have a broad family history of LS-associated cancers that co-segregate with a MMR gene mutation and microsatellite instable (MSI) tumors that lack expression of one of the DNA MMR proteins. There are however also many suspected-LS patients for whom co-segregation and tumor pathology data are unreliable. In such cases genetic screening may reveal whether the patient carries a MMR attenuating mutation. In fact, detection of a pathogenic germ-line mutation affecting one the DNA MMR genes is mandatory to establish a definitive LS diagnosis (9). Many LS-associated sequence variants are nonsense and frameshift mutations that clearly truncate the protein and unambiguously attenuate MMR. Missense mutations that alter a single amino acid are also frequently observed in suspected-LS patients (10). Without clear functional data, the diagnosis of these patients is difficult because it is not evident whether such missense mutations abrogate MMR and contribute to cancer risk. To facilitate the diagnosis of LS patients, techniques must be in place that interrogate the functional implications of MMR gene variants of uncertain significance (VUS) (11).

In the present thesis we divulge a tool for the characterization of DNA MMR gene variants.

In *Chapter 1* we provide a comprehensive overview of the DNA MMR system and how loss of DNA MMR activity predisposes to cancer. Furthermore, we describe the clinical management of LS and highlight the importance of a correct diagnosis.

In *Chapter 2* we reveal the oligonucleotide-directed mutation screen we created for the identification of pathogenic *MSH2* DNA MMR gene variants. The screen was developed in mouse embryonic stem cells (mESCs) with a single endogenous *Msh2* allele. Introduction of the desired *Msh2* sequence variant into the single functional *Msh2* allele gave rise to expression of solely the variant protein and enabled immediate investigation of its pathogenic phenotype. To determine the MMR attenuating effect of the introduced variant, cells were exposed to the DNA damaging agent 6-thioguanine (6TG). DNA MMR-deficient cells are 6TG-resistant while MMR-proficient cells die in response to 6TG exposure. Hence, solely mESCs expressing MMR abrogating variants should survive the 6TG selection. In a proof of principle study with 12 known pathogenic

mutations and 10 non-pathogenic variants, we demonstrated the ability of the genetic screen to distinguish (weak and strong) pathogenic *MSH2* variants from polymorphisms. Subsequently, we used it to analyze the functional implications of 59 *MSH2* VUS found in suspected-LS patients. Nineteen of the 59 VUS were found to lead to 6TG resistance; functional assays confirmed all 19 variants indeed abrogate MMR.

In *Chapter 3* the oligonucleotide-directed mutation screen was adapted for the characterization of *MSH6* DNA MMR gene variants. After demonstrating the screen can effectively identify pathogenic from non-pathogenic *MSH6* variants, we used it to investigate the MMR abrogating effect of 26 clinically relevant *MSH6* VUS. Of the 26 variants, 8 were identified as pathogenic. The results from our screen were compared to the clinical and tumor pathology data that were collected for these variants from medical centers in the Netherlands and literature.

In *Chapters 4* we extended the applicability of the oligonucleotide-directed mutation screen to the characterization of *MLH1* DNA MMR gene variants. We validated this approach is capable of distinguishing MMR attenuating *MLH1* mutations from polymorphism, and used it to determine the pathogenic phenotype of 50 *MLH1* VUS. Twenty-six of the 50 VUS were found to lead to MMR-deficiency.

In *Chapter 5* the genetic screen for the identification of pathogenic *MSH2* and *MLH1* variants, described in Chapters 2 and 4, was used to investigate the functional consequences of 18 VUS that were found in the *MLH1* and *MSH2* genes of 21 suspected-LS families from the Erasmus Medical Center Rotterdam and Netherlands Cancer Institute Amsterdam. This study demonstrates the clinical benefit of the genetic screen. Furthermore it proposes the relative simplicity as well as ability to study many variants in parallel makes the genetic screen a good tool for implementation in clinical genetics laboratories that are confronted with suspected-LS patients carrying MMR gene VUS.