Hereditary Colorectal Cancer; Identification, Characterization and Classification of Mutations

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Hereditary factors are thought to play are role in 20-30% of all colorectal cancers Around 6% are found as high penetrant disease-causing mutations in genes correlated to hereditary polyposis or hereditary non-polyposis syndromes. The aim of this thesis was to identify new causative genes and variants and also mutation mechanisms in families presenting a polyposis, atypical polyposis or non-polyposis CRC phenotype.

In classical familial adenomatous polyposis (FAP) 100% of the disease-causing mutations were found in patients from the Swedish Polyposis Registry. The mutation underlying the lowered expression of the *APC* gene in one family was identified by SNP array analysis, the mutation was a split deletion of 61Kb including half of the promoter 1B. Investigation of the significance of this promoter for expression of the *APC* gene demonstrated considerably higher expression compared with the established promoter 1A.

In order to establish a sensitive method for mosaic-mutation detection a comparison of mutation detection methods was performed. Low frequency mosaic mutations were detected down to 1 % by use of massively parallel sequencing (MPS). Whole exome sequencing in four families with attenuated FAP (AFAP), atypical polyposis or non-polyposis syndromes identified two high penetrant disease-causing mutations. One was found in the upstream regulatory region of *GREM1*and one in the exonuclease domain of *POLE*. Variants in low-penetrant genes possibly contributing to CRC development were also proposed from the exome sequencing and gene specific analyses of 107 patients. Sixty-seven of these patients were analyzed in a panel of 19 selected CRC predisposing genes. Truncating mutations were found in the *BMPR1A* and *SMAD4* genes in patients with a classical FAP, atypical FAP or non-polyposis phenotype. Classification of found non-synonymous variants was also performed.

In summary, using a combination of different molecular screening techniques, 100% of disease-causing mutations in classical FAP can be found. With MPS it is possible to detect low-frequency mosaic mutations down to 1% by absolute quantification. Whole exome analyses identified mutations in the new causative genes *POLE* and *GREM1*. It was also concluded that patients without identified mutations, based on phenotypical CRC classification, can have mutations in genes not included in the primary routine analysis. These results will lead to improved mutation detection analysis for diagnostic and carrier testing.

Keywords: Hereditary colorectal cancer, FAP, AFAP, atypical polyposis, mutation, PPAP APC, POLE, GREM1, exome sequencing, massively parallel sequencing, mosaic mutations

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I. Kanter-Smoler G, Fritzell K, **Rohlin A**, Engwall Y, Hallberg B, Bergman A, Meuller J, Grönberg H, Karlsson P, Björk J, Nordling M**.** Clinical characterization and the mutation spectrum in Swedish adenomatous polyposis families. *BMC Med. 2008 Apr 24;6:10*.

II. **Rohlin A**, Engwall Y, Fritzell K, Göransson K, Bergsten A, Einbeigi Z, Nilbert M, Karlsson P, Björk J, Nordling M. Inactivation of promoter 1B of APC causes partial gene silencing: evidence for a significant role of the promoter in regulation and causative of familial adenomatous polyposis. *Oncogene. 2011 Dec 15;30(50):4977-89*.

III. **Rohlin A**, Wernersson J, Engwall Y, Wiklund, L, Björk J, Nordling M Parallel sequencing used in detection of mosaic mutations: comparison with four diagnostic DNA screening techniques. *Hum Mutat Jan 30:1012-1020, 2009.*

IV. **Rohlin A**, Eiengård F, Lundstam U, Zagoras T, Nilsson S, Edsjö A, Pedersen J, Svensson JH, Skullman S, Karlsson GB, Björk J, Nordling M. Whole exome sequencing in hereditary colorectal cancer syndromes. Identification of causative mutations and contributing variants. *Submitted Manuscript*

V. **Rohlin A**, Zagoras T, Nilsson S, Lundstam U, Wahlström J, Hultén L, Martinsson T, Karlsson GB, Nordling M. A mutation in POLE predisposing to a multi-tumor phenotype. *Int J Oncol. 2014 Jul;45(1):77-81.*

VI. **Rohlin A**, Rambech E, Kvist A, Eiengård F, Wernersson J, Lundstam U, Zagoras T, Törngren T, Borg Å, Björk J, Nilbert M, Nordling M. A validated multigene panel for colorectal cancer syndromes *Manuscript*

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