Modifier Genes in Lynch Syndrome: Functional Genomics and its Consequence on Disease Expression

Stuart Reeves
B.Sc (Hons)
The University of Newcastle

Doctor of Philosophy (Medical Genetics)
The University of Newcastle
October 2011

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

28-March-2012

Date Stuart Reeves

Acknowledgements

First and foremost I would like to thank my supervisor, Professor Rodney J Scott. I am sincerely appreciative for all the support, guidance and, confidence he has provided me throughout the duration of this project. To have a supervisor of his knowledge and understanding has been an enormous privilege and something I'm very thankful for.

I'd also like to thank Dr Cliff Meldrum for his technical support and guidance. The help he kindly provided throughout the experimental stages of this project have been invaluable.

Thank you to the members of the Information Based Medicine group in particular to David Mossman for his friendship along the journey of the project. I'd also like to thank Kim Colyvas for his statistical assistance in making sense of the mountains of data generated throughout this project. A special mention needs to be made to the members of our collaboration groups who kindly provided many of the precious samples that were utilised throughout this study.

I'd like to thank the staff in the Molecular Genetics laboratory at the Hunter Area Pathology Service for making me feel welcome and the sharing of their specialist lab knowledge with me.

Finally, a big thank you must go to all my family and friends. I could have not done without your tireless encouragement and support throughout this journey.

Contents

Common Abbreviations

CRC	Colorectal Cancer
DNA	Deoxyribonucleic Acid
DNMT3B	DNA Methyltransferase 3 Beta
HNPCC	Hereditary nonpolyposis Colorectal Cancer
hMLH1	Human MutL Homolog 1
hMSH2	Mut S Homolog 2
IGF-1	Insulin like Growth Factor 1
MMR	Mismatch Repair
MTHFR	Methylenetetrahydrofolate Reductase
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
SNP	Single Nucleotide Polymorphism

Publications

1. Reeves S, Meldrum C, and Scott, R.J., *IGF-1 gene polymorphism and risk for hereditary nonpolyposis colorectal cancer.* J Natl Cancer Inst 2006; Nov 15; 98 (22): 1664-5.
2. Reeves S.G, Rich D, Meldrum C, Colyvas K, Kurzawski G, Suchy J, Lubinski, & Scott, R.J., *IGF-1 is a modifier of disease risk in hereditary non-polyposis colorectal cancer.* Int J Cancer. 2008 Sep 15; 123(6):1339-43.
3. Reeves S.G, Meldrum C, Groombridge C, Spigelman A.D, Suchy J, Kurzawski G, Lubinski J, McElduff P, & Scott, R.J., *MTHFR 677 C>T and 1298 A>C polymorphisms and the age of onset of CRC in hereditary nonpolyposis CRC.* The European Journal of Human Genetics 2009 17, 629–635.
4. Reeves SG, Mossman D, Meldrum CJ, Kurzawski G, Suchy J, Lubinski J & Scott RJ., *The -149C>T SNP within the ΔDNMT3B gene, is not associated with early disease onset in hereditary non-polyposis colorectal cancer.* Cancer Lett 2008 June 28; 265(1):39-44.
5. Reeves S.G, Meldrum C, Groombridge C, Spigelman A.D, Suchy J, Kurzawski G, Lubinski J, & Scott, R.J., *DNA repair gene polymorphisms and risk of early onset colorectal cancer in Hereditary Nonpolyposis Colorectal Cancer* – Cancer Epidemiology (Article in press October 2011)

Abstract

Colorectal cancer (CRC) is globally a major cause of morbidity and mortality. Each year more than one million patients will be diagnosed with colorectal cancer, with about 15 - 20% of these patients having a family history or an inherited colorectal cancer syndrome. Somewhere between 1% and 7% (dependent on population under study) of these cases will have Lynch syndrome, which is the most common hereditary autosomal-dominant inherited cancer syndrome caused by germline mutations in deoxyribonucleic acid (DNA) mismatch repair genes.

Patients diagnosed with Lynch syndrome who harbour a confirmed germline mutation in DNA mismatch repair (MMR) genes have an 80% lifetime risk of developing an epithelial malignancy. Each patient belongs to a family that requires special medical attention including genetic counselling, DNA testing for mismatch repair genes (most frequently *hMLH1* or *hMSH2*) and screening for CRC.

There is, however, considerable variation in the age of disease onset which is explained by a combination of genetic and environmental factors. The studies described in this thesis are aimed to better understand the genetic modifying effects on disease expression and how they relate to the likely age of colorectal cancer onset.

Previous studies have identified a polymorphic CA repeat region in *IGF-1* and two specific single nucleotide polymorphisms in MTHFR that were thought to alter the age of disease onset in individuals with Lynch syndrome. The effects of these

polymorphisms were examined in larger multinational cohorts of patients and found to have significant effects on disease onset age. This is discussed in chapters 2, 3 and 4.

Another similar study had identified a single nucleotide polymorphism in DNMT3B which was reported to have a significant effect in colorectal cancer expression in Lynch syndrome. The effect of this polymorphism was examined in a large multinational cohort of patients however, it was found to have no effect on the age of disease onset. Several candidate polymorphisms were also identified in the DNA repair genes *BRCA2*, *hMSH3*, *Lig4*, *hOGG1*, *XRCC1*, *XRCC2* and *XRCC3* but no significant associations were identified. The results from these studies are discussed in chapters 5 and 6.

All data generated from these studies were extensively analysed by a combination of statistical tests that included Kaplan-Meier survival and Cox hazard regression analysis allowing data to be stratified by both single and multi variable factors. Allele frequencies were also tested for significant deviation from the Hardy-Weinberg equilibrium, while Pearson's Chi-square test was utilised to evaluate differences in the allele frequencies between the multinational cohort groups and distribution of genotypes.

The results described in this thesis contribute to a better understanding of disease expression in Lynch syndrome as it identifies genetic factors involved in the etiology of malignancy in this disease. The progress made in this area of medical research will aid in providing better predictive information of greater accuracy regarding the risks of colorectal cancer and enable the development of personalised cancer surveillance regimens.