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# Intron Splice Acceptor Site Sequence Variation in the Hereditary Non-polyposis Colorectal Cancer Gene *hMSH2*

**N.R. Hall, G.R. Taylor, P.J. Finan, R.D. Kolodner, W.F. Bodmer, S.E. Cottrell, I. Frayling and D.T. Bishop**

**Common but weakly penetrant mutations of certain genes may confer an increased susceptibility to colorectal cancer and account for a proportion of 'sporadic' cases. We analysed DNA from 111 colorectal cancer cases and 114 controls for a specific candidate sequence variation in the hereditary non-polyposis colorectal cancer gene *hMSH2*. The variant sequence was found in a quarter of individuals, and there was no difference between cancer cases and controls, according to age of development of cancer or presence of family history. It thus appears that this particular sequence variation is a polymorphism rather than a mutation which increases cancer susceptibility.**

**Key words: colorectal cancer, DNA mutation detection, genetic susceptibility, hereditary non-polyposis colorectal cancer, polymorphism, splice site**

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## INTRODUCTION

HIGHLY PENETRANT mutations in genes, such as the newly reported DNA mismatch repair genes *hMSH2* [1, 2] and *hMLH1* [3, 4], can now be implicated in the pathogenesis of hereditary non-polyposis colorectal cancer (HNPCC). Whilst such hereditary cases are striking, they account for only a small proportion, probably less than 5%, of the colorectal cancer incidence [5–7]. Even in 'sporadic' colorectal cancer, there is familial clustering [8–10], and a substantial proportion of the cancers might result from the action of more common but less penetrant genetic mutations [11, 12]. It is, therefore, possible that a 'minor' mutation in an HNPCC gene may increase susceptibility to colorectal cancer, with significant implications for public health owing to the greater number of cancers arising in this context.

One candidate mutation has been described in *hMSH2*. A substitution of C for T in the intronic splice acceptor site six bases upstream of position 2006 (exon 13) of *hMSH2* has been found in the germline of affected individuals in one HNPCC family, and also in the tumours (but not the germline) of two individuals without a family history [1], suggesting that it might convey a selective advantage to tumour growth. Alterations in such a region might be predicted to interfere with splicing [13] and give rise to a truncated protein or diminished synthesis of a normal protein. We have also observed the variant sequence introduced by a spouse into an *hMSH2*-linked HNPCC family [14], already known to have a germline frameshift mutation of *hMSH2* (Figure 1). Some members of this family have inherited one allele containing the mutation and the other having the variant sequence, and appear to have a more severe phenotypic

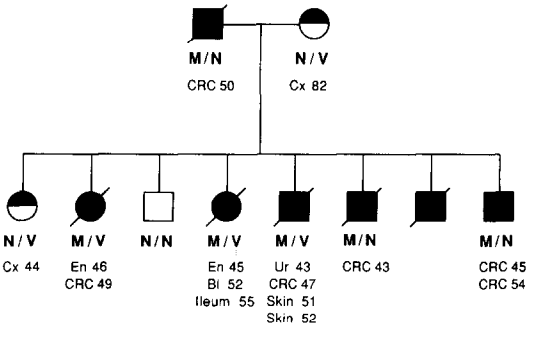


**Figure 1. Correlation of *hMSH2* genotype and phenotype in part of one HNPCC family where a mutation of *hMSH2* segregates with the HNPCC phenotype, inherited through the father. Fully shaded individuals show the HNPCC phenotype and half shading indicates a malignancy not considered part of HNPCC. Genotypes are indicated as M (mutant), N (normal) and V (variant). The mother has the variant splice acceptor sequence (V) and has passed this on to four children, all of whom have cancer. Individuals with M/V genotype appear to have a more severe phenotype, affecting organs outside the colorectum, than those with M/N genotype. Abbreviations are for carcinoma of colon/rectum (CRC), cervix (Cx), endometrium (En), bladder (Bl) and ureter (Ur). Age of onset of malignancy is shown.**

Correspondence to D.T. Bishop.
N.R. Hall and D.T. Bishop are at the Imperial Cancer Research Fund, Genetic Epidemiology Laboratory, St James's University Hospital, Beckett Street, Leeds LS9 7TF; N.R. Hall and P.J. Finan are at the Department of Surgery, General Infirmary at Leeds, Leeds LS1 3EX: G.R. Taylor is at the Regional DNA Laboratory, St James's University Hospital, Leeds LS9 7TF; R.D. Kolodner is at the Division of Cellular and Molecular Biology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115, U.S.A.; W.F. Bodmer and S.E. Cottrell are at the Imperial Cancer Research Fund, Cancer Genetics Laboratory, Lincoln's Inn Fields, London WC2A 3PX; and I. Frayling is at the Imperial Cancer Research Fund, Colorectal Unit, St Mark's Hospital, London EC1V 2PS, U.K.
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*Table 1. Genotypes of* hMSH2 *splice acceptor site*

| Splice acceptor site genotype | Patients with colorectal cancer Group 1 Age under 45 years *n* (%) | Group 2 Single surgeon *n* (%) | Controls Group 3 Spouses *n* (%) | Total *n* (%) |
|---|---|---|---|---|
| Normal/normal | 23 (72) | 59 (75) | 87 (76) | 169 ( 75) |
| Normal/variant or variant/variant* | 9 (28) | 20 (25) | 27 (24) | 56 ( 25) |
| Total | 32 | 79 | 114 | 225 (100) |

*Only one individual was identified as a homozygote variant (in group 2).

expression of HNPCC than those with the mutated *hMSH2* alone. Other workers, however, have demonstrated that this variant sequence was common in the general population, having found it in two out of 20 individuals without cancer, leading to the suggestion that it is a polymorphism rather than a disease-producing mutation [2].

We, therefore, sought to address the issue of whether the splice site sequence variation might alter the susceptibility of individuals to the development of colorectal cancer. This was achieved by comparing the frequency of occurrence of the variant sequence in colorectal cancer patients to that in a control group.

## PATIENTS AND METHODS

Three groups of individuals were studied: group 1 is a population-based sample of 32 cases diagnosed under the age of 45 years in Yorkshire; group 2 is a representative selection (79 in total) of cases of all ages presenting to a single surgeon and group 3 is a control group of 114 spouses of cancer cases. In 92 patients, a validated family history was available: 72 had no first degree relatives with colorectal cancer and 20 had one or more first degree relatives affected.

We utilised a fluorescence-based modification of the amplification refractory mutation system (ARMS) principle [15] specifically to detect the T to C transition in a single polymerase chain reaction. The downstream primer sequences differed only by a single base at the 3′ end to match the normal or variant sequence CTTGCTTTCTGATATAATTTGTTT/C, and each was attached to a different colour fluorescent dye. A 285 base pair fragment of the gene was amplified (27 cycles with an annealing temperature of 57°C) in a 20-μl reaction using 5 pmol normal sequence primer, 10 pmol variant sequence primer and 10 pmol of a common unlabelled primer (primer 16340 [1]) to initiate the upstream fragment. The reaction conditions were optimised to allow ease of interpretation of results. Products were analysed using denaturing polyacrylamide gel electrophoresis and Applied Biosystems 672 Genescan software (Applied Biosystems Inc., Warrington, U.K.). The relative amplification of each coloured fluorescent product enabled classification of genotype as normal/normal, normal/variant or variant/variant. Validation of our technique has been provided by direct sequencing [1] and single strand conformation polymorphism analysis in a sample of individuals.

## RESULTS

An equal proportion of each of the three groups (about 25%) was heterozygous at the *hMSH2* splice acceptor site ($\chi^2 = 0.28$, $P = 0.87$, Table 1). Family history information was available for 92 of the 111 patients: of the 72 individuals with no family history of colorectal cancer, 23 (32%) were heterozygous for the variant sequence, and of the 20 cases where one or more first degree relatives had verified colorectal cancer, 2 (10%) had the variant sequence ($\chi^2 = 3.8$, $P = 0.051$). The individual with the homozygous variant sequence developed colorectal cancer aged 56 and had no family history of cancer.

## DISCUSSION

There was no significant difference in the prevalence of the splice acceptor site sequence variation between cases and controls. Analysis according to family history suggested, if anything, a trend towards a protective rather than detrimental effect of the variant sequence, though this was of borderline significance. We conclude that the T to C sequence variation is a common polymorphism with an allele frequency of about 1 in 8, and does not, in fact, increase susceptibility to the development of sporadic colorectal cancer. Our study was designed to examine the variant sequence in the context of 'sporadic' colorectal cancer. Although we consider it unlikely, it remains possible that the variant could modulate the effect of a more penetrant *hMSH2* mutation in HNPCC families where (such as demonstrated in the figure) the two sequences are present in the same individual.


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# Tissue-specific Expression of Neural Cell Adhesion Molecule (NCAM) May Allow Differential Diagnosis of Neuroblastoma From Embryonal Rhabdomyosarcoma

E.G. Phimister, A. Culverwell, K. Patel and J.T. Kemshead

**The MSD1 region of neural cell adhesion molecule (NCAM) was originally described as being spliced into the 120-kDa isoform of NCAM isolated from muscle. The 105 bp region is inserted between exons 12 and 13 and actually consists of three separate exons, MSD1a, MSD1b and MSD1c of 15, 48, 42 bp, respectively. In addition, a further exon consisting of a single triplet has been designated MSD1d, making the full insert size 108 bp. As the MSD1 region was originally described as being selectively expressed in muscle tissue, we have investigated whether it is also present on tumours of rhabdoid origins and whether its presence can be used as the diagnostic marker to distinguish other small round cell tumours of childhood, such as neuroblastoma. Using a variety of human tumour cell lines, we demonstrated the presence of the MSD1 region on all rhabdomyosarcomas investigated. However, neuroblastoma cell lines only expressed subcompartments of the MSD1 region. The MSD1c exon was not spliced into the NCAM molecules isolated from any of the neuroblastoma cell lines investigated. On the basis of this finding, it appears that neuroblastoma and rhabdomyosarcoma can be distinguished by the expression of the MSD1c mini-exon. Further studies are underway to attempt to define a monoclonal antibody that recognises the region, using mice immunised with synthetic peptides, and to confirm the finding using fresh biopsy material.**
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## INTRODUCTION

NEUROBLASTOMA IS a malignancy of childhood with an incidence of approximately one child in 10 000 between the ages of birth and 14 years [1]. Along with Wilms' tumour, Ewing's sarcoma, rhabdomyosarcoma and lymphoblastic leukaemia/lymphoma, it represents one of the small round cell tumours of childhood. Whilst these can often be differentiated on the basis of clinical presentation, pathology and a variety of biochemical investigations, it can prove difficult to distinguish one tumour type from another, particularly when the cells are highly anaplastic in nature.

As the therapy for the small round cell tumours of childhood differs for each malignancy, a concreted effort has been made to ensure an accurate diagnosis of tumour type. Various biochemical studies can be undertaken as an aid to identifying a particular tumour, such as the presence of catecholamine breakdown products in the urine of patients with neuroblastoma [2]. The fact that these cells often synthesise catecholamines can also be exploited in diagnostic imaging through the use of *meta*-iodobenzylguanidine (*m*IBG), a synthetic analogue of adrenaline and nor-adrenaline. This can be radiolabelled with an isotope of iodine, and it will accumulate *in vivo* in the majority of neuroblastomas investigated, allowing their identification by scintigraphy [3].

Correspondence to J.T. Kemshead.
All authors are at the Imperial Cancer Research Fund, Paediatric & Neuro-Oncology Group, Frenchay Hospital, Bristol BS16 1LE, U.K.
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