

# **BAT26 Microsatellite Marker Polymorphism in Colorectal Cancer in Senegalese Patients**

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**Abstract:** Colorectal cancer is a serious problem in cancerology due to its frequency and severity. It is the third most common form of cancer, with one million new cases worldwide each year, and its mortality is around 30%. Despite significant advances in "omics" techniques that allow us to understand the molecular mechanisms involved in the evolution of cancer pathologies, the strongest risk factors currently involved in the etiology of this cancer are those of a genetic nature, with specific mutations. The aim of this study is therefore to accurately establish the incidence of the MSI phenotype in Senegalese patients with colorectal cancer using the *BAT26* single nucleotide marker. To identify these phenotypes, the *BAT26* loci sequences of 18 cancer patients were compared with a reference sequence and their haplotypes were determined through DnaSP version 5.10. The MEGA software version 7.014 and the Arlequin program version 3.1 were used to highlight the genetic differentiation as well as the demo-genetic evolution of our study populations. The results of this comparison revealed a high microsatellite instability. This nucleotide difference is materialized by adenine to guanine substitutions present in the sequences of 17 cancer patients. This variability between cancer tissues is confirmed by the Nei genetic distance which shows that cancer tissues are genetically heterogeneous. The *Fst* indicates that there is genetic structuring between the cancer tissue sequences and our control (the reference sequence). These results suggest that this polymorphism of the *BAT26* single-nucleotide marker may be involved in the occurrence of this cancer.

**Keywords:** *BAT26*, Colorectal Cancer, Instability, Microsatellite, Variability

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## **1. Introduction**

Colorectal cancer is the second leading cause of cancer death in Western countries. Its incidence is gradually increasing in emerging countries [1]. Worldwide, it is the second and third most common cancer in women and men respectively [2]. Although there is an increase in colorectal cancer in developing countries, particularly in West Africa, data on the epidemiology and biology of colorectal cancer in this region are scarce [3]. According to Konaté and his collaborators (2012) in reference [4], they are frequently encountered in hospital practice in Senegal, contrary to the data in the literature reporting their rarity in Africa. About 15 to 20% of colorectal cancers (CRC) develop in a family context with the aggregation of several cancers in the same family [5]. But only 2 to 5% correspond to hereditary cancers, that is to say

cancers of transmission genetics, for which the responsible gene is clearly identified [6]. Among the forms of colorectal cancer, Lynch syndrome is the most common and is linked to a germline mutation of one of the DNA mismatch repair genes. This mutation is associated with an unstable tumor DNA phenotype that is reflected by the presence of supernumerary alleles in microsatellite marker genotyping [2]. It has been well documented that there are two main pathways in colorectal carcinogenesis. One is the chromosomal instability pathway, characterized by allelic losses on chromosomes 5q (*APC*), 17p (*p53*) and 18q (*DCC/SMAD4*), and the other is a pathway that involves microsatellite instability [7]. Their instability leads to the accumulation of mutations in genes controlling apoptosis and the cell cycle and is at the origin of rare genetic syndromes (FMP, HNPCC, Peutz-Jeghers syndrome) [8, 9]. Microsatellites are short tandemly repeated DNA sequences, which instability was first

described in colorectal tumors [10] consequently present in 10-15% of colorectal tumors [11]. This frequency of microsatellite instability in colorectal tumors raises the interest of conducting genetic studies in the Senegalese population. It is in the context, we aim to determine the involvement of genetic alterations of the *BAT26* single-nucleotide marker in case of colorectal cancers.

2. Methodology

2.1. Samples

The study was done on 25 colorectal cancer patients. These patients were recruited from the general surgery departments of the Aristide le Dantec hospital, the Principale hospital in Dakar, Grand-Yoff and the oncology department of the Dantec hospital. For each operated patient, a fresh surgical specimen was taken in the middle of the tumor, collected in a dry tube and stored at 20°C, along with the clinical information sheet. Samples were directly sent to the genomics laboratory of the Department of Animal Biology of the Faculty of Science and Technology of the University of Dakar where the tissues were conserved in 96% alcohol.

2.2. DNA Extraction, Amplification and Sequencing of the *BAT26* Gene

The total DNA from each sample was extracted using the Standard Zymo method (Zymo research kit). After extraction, the region spanning intron 5 of the *MSH2* gene containing the *BAT26* microsatellite marker was amplified in a reaction volume of 25 µl with primers: F 5'-TGACTACTTTTGACTTCAGCC-3') and R 5'- AACCATCAACATTTTAAACCC -3'. The PCR is performed in an Eppendorf thermal cycler with the conditions: preliminary denaturation at 95°C (5 minutes), followed by a repeat of 35 cycles of denaturation at 95°C (30 seconds), primer hybridization at 47°C (1 minute) and elongation of complementary DNA strands at 70°C for (1 minute 30s) and is closed by a final elongation cycle at 70°C for 10 minutes. To ensure that each target gene has been amplified, electrophoretic migration is performed on a 2% agarose gel. The 1977 F. Sanger

sequencing method is used to identify the nucleotide sequence of the *BAT26* locus.

2.3. *BAT26* Microsatellite Polymorphism

A difference in size and pattern of loci between cancer tissue and control is considered instability. The *BAT26* microsatellite marker phenotype of cancer tissue sequences is determined based on the number of unstable loci. The list of haplotypes was obtained through DnaSP version 5.10 software [12].

2.4. Genetic Differentiation

The estimation of genetic differentiation allows to account for the inter-tissue genetic structure. For this purpose, two parameters have been highlighted: the dissimilarity index or genetic distance and the genetic differentiation index (Fst).

The determination of the genetic distances of Nei (1978), intra and inter-populations, consists in evaluating the number of allele replacements that have occurred at the locus during evolution. It was done with MEGA software version 7.0 [13]. As for the determination of genetic differentiation (Fst) of Cavalli-Sforza (1966), it consists in measuring the variations of the allelic frequency of SNPs between tissues in order to detect positive selection events. It was done with the Arlequin program version 3.1 [14]. Its value is always between 0 (when there is no structuring) and 1 (if there is genetic structuring). A value of P < 0.05 was considered significant.

2.5. Mismatch Distribution

Mismatch distribution analysis is the graphical representation of the distribution of genetic distances between individuals in a population. It is accompanied by two indices that test the goodness of fit of the distribution. These indices are the SSD (sum of squares of deviations) and the Raggedness (Rag irregularity index). The graphs were constructed with the software DnaSP version 5.10 [12] and the SSD and Rag indices were obtained with the program Arlequin version 3.1 [14].

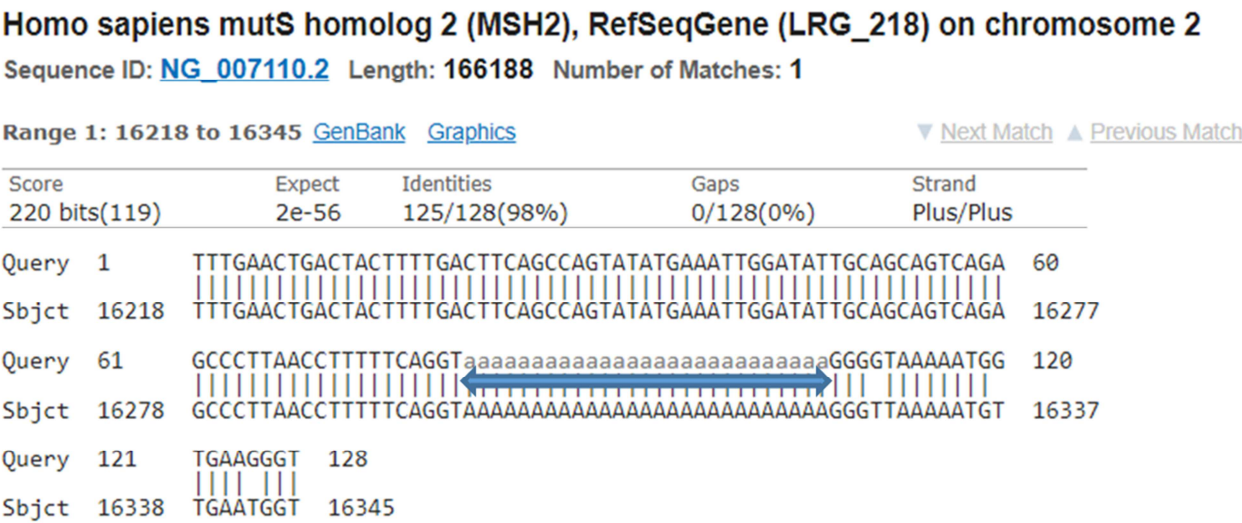


Figure 1. Position and nucleotide sequence of the *BAT26* microsatellite indicated by the blue arrow.

### 3. Results

#### 3.1. Nucleotide Sequences

After amplification on CRC tissue samples, we were left with 18 amplicons of the *BAT26* marker and 7 individuals for which PCR failed. Sequence alignment of the 18 amplicons with a reference sequence obtained after blast (Figure 1) shows the adenine repeat of the *BAT26* microsatellite from position 16298 at the chromosomal level.

#### 3.2. *BAT26* Microsatellite Polymorphism in Cancer Tissues

A difference in size and pattern was noted on the marker studied in CRC patients. Of the 18 tumors analyzed, 17 (94.44%) were found to be unstable. In these 17 samples, large substitutions of adenine in guanine were noted and for one sample no mutation was observed compared to the control sequence consisting of a 27 A (Adenine) repeat. The *BAT26* instability of our samples is characterized by 4 motifs represented in Table 1. Based on the microsatellite reference pattern in the 27A form, 5 haplotypes were found in our 18

CRC patients (Table 2). These haplotypes also indicate a variation in the number of adenine repeats ranging from 23 to 27 (Figure 2). Among the 5 haplotypes, haplotypes one (H1), two (H2) and five (H5) show adenine to guanine substitutions at respective positions 16323A>G found in 14 cancer tissue sequences representing 77.78%, 16322-16324A>G (5.56%) and 16322A>G; 16323A>G (5.56%). These substitutions were also found on haplotype three (H3) in addition to a substitution of the last adenine of the thymine repeat at the following positions: 16309A>G; 16322A>G; 16323A>G and 16324A>T with a frequency of (5.56%). The haplotype (H4) has no microsatellite mutations compared to the reference and also accounts for (5.56%). These results are presented in table 2.

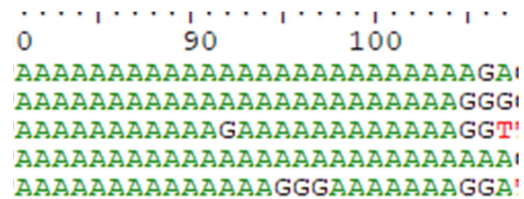


Figure 2. *BAT26* microsatellite length polymorphism in colorectal cancer.

Table 1. Different motifs of the *BAT26* marker in cancer tissues compared to the reference.

Locus <i>BAT26</i>	Patterns	Haplotypes	Number of CT
Reference	AAAAAAAAAAAAAAAAAAAAAAAAAAAAA	27 A	1
	AAAAAAAAAAAAAAAAAAAAAAAAAAAAA	27 A	1
	AAAAAAAAAAAAAAAAAAAAAAAAAAGA	25A Sub A <sub>26</sub> →G	14
Cases	AAAAAAAAAAAAAAAAAAAAAAAAAAGGG	24A Sub A <sub>25_26_27</sub> →G	1
	AAAAAAAAAAGAAAAAAAAAAGGT	23A Sub A <sub>12_25_26</sub> →G; A <sub>27</sub> →T	1
	AAAAAAAAAAGGGAAAAAAAAAGGA	Sub A <sub>15_16_17_25_26</sub> →G	1

A = Adenine, G = Guanine, Sub = Substitution, CT = Cancerous tissue.

Table 2. Length and pattern polymorphism of repeated nucleotide of intron 5 of *MSH2* gene in colorectal cancers.

Haplotype	Number (%)	Microsatellite pattern	Variants
H1	14 (77,78%)	A <sub>25</sub> GA	16323A>G
H2	1 (5,56%)	A <sub>24</sub> GGG	16322-16324 A>G
H3	1 (5,56%)	A <sub>11</sub> GA <sub>12</sub> GGT	16309A>G; 16322A>G; 16323A>G; 16324A>T
H4	1 (5,56%)	A <sub>27</sub>	wide type
H5	1 (5,56%)	A <sub>14</sub> GGGA <sub>7</sub> GGA	16322A>G; 16323A>G

#### 3.3. Genetic Differentiation

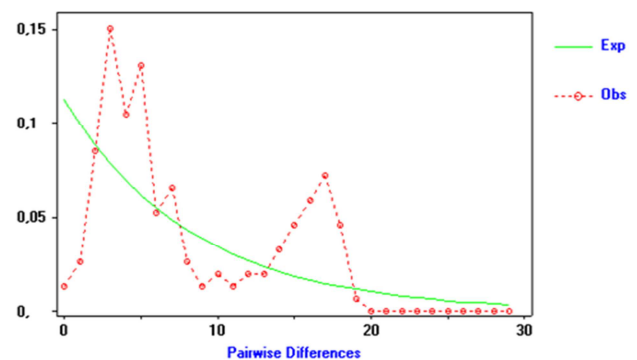
Genetic distance analysis revealed low intra-tissue genetic distances of 0.076 at the cancer tissue level and a low inter-tissue genetic distance of (0.074) of the *BAT26* microsatellite. A significant *Fst* of 1 for the microsatellite is obtained with a zero *p* value. All of these results are presented in Table 3.

Table 3. Intra- and inter-group genetic distances and the genetic differentiation between all pairs of samples.

Genetic distances		
Groups Sequences	Intra-groupe	Inter-groupe
SR	-	0,074 (0,023)
CT	0,076 (0,019)	
Genetic differentiation ( <i>Fst</i> )		
SR	1.00000 (0.0000)	
CT		

SR = Reference sequence; CT = Cancerous tissue.

#### 3.4. Mismatch Distribution Analysis



SSD = 0.0174 (*P* = 0.3700); Rag = 0.0252 (*P* = 0.3800)

Figure 3. *BAT26* mismatch distribution.

The mismatch distribution of the loci base pairs shows

the expected and observed frequencies (solid and dashed lines, respectively) of pairwise differences between samples. The results show multimodal distributions (Figure 3). This plot is supported by the SSD and Rag indices, which are not significant for the cancer population indicating that there is no discrepancy between the observed and expected values.

## 4. Discussion

The objective of this study is to determine the involvement of genetic alterations of the part of intron 5 of the *MSH2* gene containing the *BAT26* microsatellite marker in the evolution of colorectal cancers in the Senegalese population. Cancers being governed by the mutation-selection process specific to our evolution, which allowed us to better understand the history of tumor cells and to set a number of specific objectives among which genetic differentiation, genetic evolution at the level of colorectal tissue sequences. In the case of this study, we undertook the analysis of the *BAT26* loci sequences of 18 Senegalese patients with colorectal cancer, compared to a reference sequence obtained in GenBank.

Our results showed that the polymorphism of the *BAT26* marker was found to be highly variable. It is manifested by substitutions of a variable number of adenine to guanine in the majority of the cancer tissue sequences analyzed and a substitution of adenine to thymine on one of the sequences resulting in a shortened size compared to the reference. This polymorphism is consistent with that obtained with haplotypes where variations in the number of adenine repeats ranging from 23 to 27 are observed. Of the 5 haplotypes, H1, H2, H3, and H5 have adenine to guanine substitutions at respective positions and frequency: 16323A>G found in 14 cancer tissue sequences (77.78%), 16322-16324A>G (5.56%), 16309A>G; 16322A>G; 16323A>G (5.56%) and 16322A>G; 16323A>G. Only H3 has in addition to adenine to guanine changes of an adenine to thymine substitution at position 16324A>T. Haplotype 4 has no mutation compared to the reference. Indeed, expression of mismatch genes measured by immunohistochemistry showed that in simple MSI-H cancers failed to express *MLH1* or *MSH2* in seven out of nine cases (78%) and MSI-L/MSS cancers failed to express *MLH1* or *MSH2* in one out of 45 cases (2.2%) [15]. According to Duval and Hamelin in reference [16], these genes are called target genes of the instability of cancers with alteration of the MMR system and that their responsibility in MSI-H carcinogenesis, due to the instability of these repeats, has been recently demonstrated.

These results are similar to those of [17] in their study on the evaluation of the *BAT25* and *BAT26* polymorphism in Senegalese subjects with colorectal cancer. In this study most of the mutations found were adenine to cytosine substitutions in the tumor tissues, and an adenine to guanine substitution for the sequence of one sample. A panel of 3 dinucleotide markers as well as 5 microsatellites including *BAT26* was proposed at the International Consensus

Meeting on MSI Frequency in Various Human Tumors held in Bethesda in December 1997 [9]. The work of Pyatt and his collaborators (1999) in reference [18], which highlights the natural polymorphisms of this marker in Africans, shows that the use of this single-nucleotide repeat microsatellite for MSI characterization has proven to be advantageous over many di-, tri-, or tetra-nucleotide repeat microsatellites due to its near-single-nucleotide nature and sensitivity to MSI. Further studies with a much larger sample size might lead us to believe that the observed mutations have a causal role in the disease. As in these studies where the *BAT26* locus showed reduced adenine repeats in 18 of 19 colorectal tumors [19], in 100% of 27 cell lines [20] and in 41 of 42 colorectal tumors and cell lines [10]. This same phenomenon was found in other pathology for example on The analysis of 78 gliomas, in 31 thyroid cancers [21] presenting a *BAT26* allele shortened by 7 or 12 bases in the tumor and in the analysis of 60.71% of tissues affected by breast cancer were found to be unstable presenting clinicopathological impacts and survival (P: 0.0342) of Senegalese patients according to Mbaye and his colleagues in reference [22]. The study of the genetic structure is performed between the sequences of cancerous tissues and reference sequence. The analysis of their genetic distance and the genetic differentiation index (*Fst*), shows an incipient genetic differentiation characterized by a low inter-tissue genetic distance of (0.074) of the microsatellite and a very significant *Fst* with zero p-value; this reflects the malignant state of the tumor cells. These results are in line with the mismatch plot. The mismatch plot indicates a multimodal distribution of the microsatellite showing a sign of a stable population.

## 5. Conclusion

The results of our analyses of the *BAT26* microsatellite marker length polymorphism in Senegalese patients with colon and rectal cancer showed that there is variability between the reference sequence and the tumor tissue with different mutations from one sequence to another. This variable is confirmed by the intra-individual genetic distances. This variable expressivity of the *BAT26* loci confirms their involvement in the occurrence of this cancer. Although there are several reports of mutations of the studied markers in various tumors of different anatomical origin, the functional effect of these in metabolism and cell growth is not clearly elucidated. Thus, as this study has a predictive role, our results pave the way for future biochemical, proteomic and clinical studies to determine the effect of these somatic mutations in the energy metabolism of tumor cells. Furthermore, the understanding of the mechanisms involved in the racial variation of the prevalence of this disease should be investigated by trying to enlarge the sample size and to include all the clinicopathological parameters of the disease but also to take into account the lifestyle of the patients.

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