

Detection of Heterozygous Mutations in Hereditary Colorectal Cancer Using the BESS-T™ Base Reader Kit

Angela Brieger,¹ Jörg Trojan,¹ Jochen Raedle,¹ W. Kurt Roth,² and Stefan Zeuzem¹

¹Medizinische Klinik II, Klinikum der Johann Wolfgang Goethe-Universität

²Blutspendedienst Hessen, D-60590 Frankfurt a.M., Germany

Introduction

Inheritance of defective DNA mismatch repair genes is associated with hereditary nonpolyposis colorectal cancer (HNPCC). Defects in hMLH1 and hMSH2 account for approximately 98% of mutations found in HNPCC families.¹ Genetic diagnosis of at risk individuals within these families is critical for effective surveillance. Characterization of mutations by direct sequencing is time-consuming and not feasible in a large scale clinical setting. Molecular screening strategies, including single-strand conformation polymorphism (SSCP) analysis,² denaturing gradient-gel electrophoresis (DGGE),³ constant denaturant gel electrophoresis (CDGE),⁴ or *in vitro* transcription/translation (IVTT) assay,⁵ have been described and may facilitate the detection of mutations. However, these techniques often provide low sensitivity (mutation detection rates of only 35-70%)⁶ or they are highly accurate but technically difficult.³⁻⁵

In the present study, we evaluated Base Excision Sequence Scanning (BESS)⁷ using the BESS-T Base Reader Kit as a method for detecting hMLH1 and hMSH2 germline mutations. This simple method detects mutations by incorporating limiting amounts of dUTP into a PCR product. Subsequent enzymatic treatment of the PCR product results in removal of uracil and cleavage of the phosphodiester bonds at these sites to produce a set of nested fragments similar to a "T"sequencing ladder.

BESS analysis identifies missense mutations, deletions, insertions, repeat expansions and frame shift mutations. Mutations at sites involving dTTP account for 96% of known hMLH1 and hMSH2 mutations.^{1,9} To date only 8 of 208 hMLH1 and hMSH2 mutations are G→C or C→G missense mutations.⁹ Thus, the sensitivity of BESS-T analysis to detect hMLH1 and hMSH2 mutations is higher than the IVTT (62%),⁵ and SSCP (35-70%) techniques.⁶ (Editor's note: EPICENTRE's BESS-G Base Reader Kit can detect mutations at sites involving guanine.)

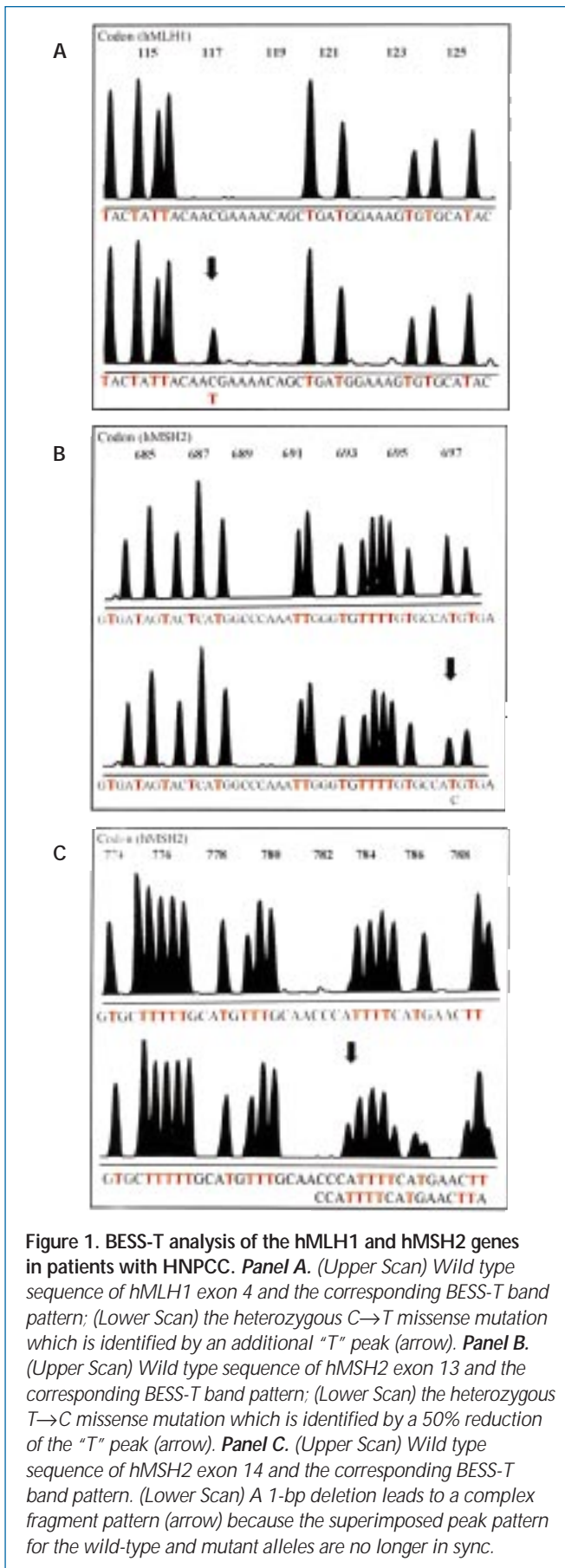
Methods

Lymphocytes were prepared from whole blood of patients with HNPCC and healthy subjects using Vacutainer cell preparation tubes. After extraction of total RNA complementary DNA synthesis was performed with reverse transcriptase and random hexamer oligonucleotides or 2.5 μM reverse primers.* The PCR amplification was carried out in a total volume of 50 μl, containing 5 U *Taq* DNA polymerase, 60 mM Tris-HCl (pH 8.5), 15 mM (NH₄)₂SO₄, 3.5 mM MgCl₂, 200 μM of each dNTP plus 16 μM dUTP, and 2.5 μM forward and reverse primer.* Either the forward or the reverse primer

was labeled with 6-carboxy-fluorescein (FAM). Amplification conditions were as follows: 10 minutes at 95°C; 50 cycles of 30 seconds at 95°C, 30 seconds at 50°C, 55°C or 65°C depending on the amplified fragments,* and 1 minute at 72°C; final extension 10 minutes at 72°C. Gel purified PCR products were digested in 20 μl of a solution with 2 μl BESS-T Excision Enzyme Mix, 50 mM Tris HCl (pH 9.0), 20 mM (NH₄)₂SO₄ and 10 mM EDTA at 37°C for 45 min. The fragments of the digested PCR products, ranging in size from 22 to 480 bp, were mixed with 11.5 μl formamide and 1.5 μl TAMRA size standard (N,N,N',N' tetramethyl-6-carboxyrhodamine) (Perkin Elmer) and electrophoresed for 40 min on an automated ABI 310 DNA sequencer with laser scanning and linear detection characteristics (Perkin Elmer). BESS-T analyses of the hMLH1 and hMSH2 genes were performed in 4 patients with known sequence-confirmed mutations and 4 at risk patients, who fulfilled the Amsterdam criteria and revealed microsatellite instability. Electropherograms were assessed by an investigator who was unaware of the direct sequence data. All patients consented to participate in the study, which was approved by the Ethics Committee for Medical Research in Frankfurt a. M. in accordance with the Declaration of Helsinki. (*Refer to "Brieger, A. *et al.* (1999) *Clin. Chem.* **45**, 1564" for primer sequences.)

Results and Discussion

In families with HNPCC, 214 different germline mutations have been described by direct sequence analysis: 127 mutations were located in the hMLH1 gene and 81 in the hMSH2 gene, whereas only 6 mutations were detected in either the MSH6, the PMS1 or the PMS2 gene.⁹ To establish and evaluate the BESS-T Base Reader Kit as a screening method, three representative mutations and one polymorphism of hMLH1 and hMSH2 were analyzed. Initially, we investigated a heterozygous ACG→ATG missense mutation in codon 117, exon 4 of the hMLH1 gene leading to a change from threonine to methionine (hMLH1, Thr117Met). BESS analysis clearly identified this mutation by an additional peak at nucleotide position 350 (Figure 1A). In addition, we performed BESS-T analysis in a patient with a heterozygous TGT→CGT missense mutation in codon 697, exon 13 of the hMSH2 gene (hMSH2, Cys697Arg). As shown in Figure 1B, this mutation was detectable by a 50% reduction of the "T"-peak at nucleotide position 2089. As a third representative mutation, we investigated an unidentified 1 bp deletion within codon 782, exon 14 of the hMSH2 gene (hMSH2, DEL782FS). This frameshift mutation lead to a complex fragment pattern in the BESS-T analysis, which is caused by the superimposed band pattern of the



wild type and mutant alleles. When compared with the BESS-T pattern of a healthy control subject, the deletion at nucleotide position 2345 was clearly identified (Figure 1C). However, in a patient with a known GGC→GGG polymorphism in codon 713 of the hMSH2 gene (hMSH2, Gly713Gly), a BESS-T pattern identical to healthy control subjects (n=7) was observed (data not shown).

Subsequently, we investigated the hMLH1 and hMSH2 genes of 4 patients fulfilling the Amsterdam criteria prospectively by the BESS protocol. In two patients missense mutations in the hMSH2 gene, one in codon 322 (hMSH2, Gly965Asp) and one yet not described mutation in codon 388 (hMSH2, Pro1165Leu), were detected. In the third patient, BESS-T analysis showed another yet not described missense mutation in codon 618 of the hMLH1 gene (hMLH1, Glu1853Asp). All mutations detected by the BESS-T protocol were confirmed by direct sequencing. In the remaining patient, BESS-T analysis and direct sequencing revealed no mutation in hMLH1 and hMSH2.

References

1. Peltomäki, P. and Vasen, H.F. (1997) *Gastroenterology* **113**, 1146.
2. Orita, M. *et al.* (1989) *Genomics* **5**, 874.
3. Wijnen, J. *et al.* (1995) *Am. J. Hum. Genet.* **56**, 1060.
4. Borresen, A.L. *et al.* (1995) *Hum. Mol. Genet.* **4**, 2065.
5. Luce, M.C. *et al.* (1995) *Gastroenterology* **109**, 1368.
6. Sarkar, G. *et al.* (1992) *Nucleic Acids Res.* **20**, 871.
7. Hawkins, G.A. and Hoffman, L.M. (1997) *Nat. Biotechnol.* **15**, 803.
8. Hawkins, G.A. and Hoffman, L.M. (1999) *Electrophoresis* **20**, 1171.
9. ICG-HNPCC database. <http://www.nfdht.nl/database/mdbchoice.ht>

BESS-T™ Base Reader Kit

BN712100-F71 100 Templates
(200 BESS-T™ Rxns)

BESS-G™ Base Reader Kit

GT85100-F71 100 Templates
(200 BESS-G™ Rxns)

BESS-T&G™ Base Reader Kit

BTG8520-F71
40 BESS-T™ and 40 BESS-G™ Rxns
BTG85100-F71
200 BESS-T™ and 200 BESS-G™ Rxns
Contains reagents to fully characterize 20 or 100 templates.

For more information, please circle reader service number N716 on the reply card found in the center insert or visit our website at www.epicentre.com/catalog/bessmut.htm