GASTROENTEROLOGY 2013;145:544–547

Germline Mutations in the Spindle Assembly Checkpoint Genes *BUB1* and *BUB3* Are Risk Factors for Colorectal Cancer

RICHARDA M. DE VOER,[1,*] AD GEURTS VAN KESSEL,[1,*] ROBBERT D. A. WEREN,[1,*] MARJOLIJN J. L. LIGTENBERG,[1,2,*] DOMINIQUE SMEETS,[1] LEI FU,[3,4] LILIAN VREEDE,[1] EVELINE J. KAMPING,[1] EUGÈNE T. P. VERWIEL,[1] MARC–MANUEL HAHN,[1] MAAYKE ARIAANS,[1] LIESBETH SPRUIJT,[1] TON VAN ESSEN,[5] GUNNAR HOUGE,[6] HANS K. SCHACKERT,[7] JIAN Q. SHENG,[3] HANKA VENSELAAR,[8] CONNY M. A. VAN RAVENSWAAIJ–ARTS,[5] J. HAN J. M. VAN KRIEKEN,[2] NICOLINE HOOGERBRUGGE,[1] and ROLAND P. KUIPER[1]

Departments of [1]Human Genetics and [2]Pathology, Radboud University Medical Centre and Research Institute for Oncology, Nijmegen, The Netherlands; [3]Department of Gastroenterology, General Hospital of Beijing Military Region, Beijing, China; [4]Third Military Medical University, Chongqing, China; [5]Department of Genetics, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands; [6]Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway; [7]Department of Surgical Research, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; and [8]Centre for Molecular and Biomolecular Informatics, Nijmegen Centre for Molecular Life Sciences, Nijmegen, The Netherlands

The spindle assembly checkpoint controls proper chromosome segregation during mitosis and prevents aneuploidy—an important feature of cancer cells. We performed genome-wide and targeted copy number and mutation analyses of germline DNA from 208 patients with familial or early-onset (40 years of age or younger) colorectal cancer; we identified haploinsufficiency or heterozygous mutations in the spindle assembly checkpoint genes *BUB1* and *BUB3* in 2.9% of them. Besides colorectal cancer, these patients had variegated aneuploidies in multiple tissues and variable dysmorphic features. These results indicate that mutations in *BUB1* and *BUB3* cause mosaic variegated aneuploidy and increase the risk of colorectal cancer at a young age.

Keywords: Cancer Predisposition; Spindle Assembly Checkpoint; Mosaic Variegated Aneuploidy.

Chromosomal aneuploidies are common in cancer and can exert oncogenic effects.[1,2] The spindle assembly checkpoint (SAC) controls correct chromosome segregation by delaying anaphase onset until all chromosomes are properly attached to mitotic spindles. Studies in mice have shown that proper SAC functioning is highly dependent on the strictly orchestrated expression of its components, and that an imbalance of one of these components can lead to aneuploidies and tumor formation.[3–6] In humans, biallelic mutations in the SAC component *BUB1B* cause mosaic variegated aneuploidy (MVA) syndrome (OMIM: 257300), which is marked by developmental abnormalities, MVA, and a predisposition to develop childhood cancer.[7,8] Recently, it was found that milder *BUB1B* mutations can lead to an increased risk of developing gastrointestinal tumors.[9]

We performed high-resolution genome-wide copy number profiling in patients diagnosed with nonpolyposis, mismatch-repair proficient colorectal cancer (CRC) before or at the age of 40 years (n = 39) and identified a heterozygous germline microdeletion in chromosome 2q13 in a patient who developed CRC at the age of 37 years (patient 1; Table 1).[10] This approximately 1.75-Mb deletion encompasses 11 genes, including *BUB1* (Figure 1*A* and Supplementary Figure 1*A*). BUB1 is an integral component of the SAC, and disruption of this complex can result in chromosomal missegregation.[11,12] Cytogenetic analyses of lymphocytes and primary skin fibroblasts revealed variegated aneuploidies and structural abnormalities in 38% and 35% of the metaphases, respectively (Supplementary Figure 2*A*). Real-time quantitative polymerase chain reaction and Western blot analyses showed that BUB1 messenger RNA and protein levels were reduced by approximately 50% in lymphoblastoid cells and fibroblasts compared with age-matched controls, suggesting haploinsufficiency. Immunofluorescence analyses confirmed normal BUB1 localization at the kinetochores (Supplementary Figure 3). In addition, we identified 10 similar 2q13 microdeletions in 10,139 patients with congenital abnormalities, as described by Yu et al.[13] Cytogenetic analyses of 4 of these cases revealed low levels of MVA in 1 case and premature sister chromatid separation in another case (Supplementary Figure 4).

To reveal whether haploinsufficiency of *BUB1* is responsible for the chromosome segregation defects observed, we generated an isogenic HCT116 cell line with a monoallelic disruption of *BUB1* (Supplementary Figure 1*B* and *C*). Cytogenetic analysis of HCT116-*BUB1*$^{+/-}$ revealed a gain of one or more chromosomes in 11% (range, 47–53 chromosomes) and a loss of one or more chromosomes in 38% (range, 43–45 chromosomes) of the metaphases, which is significantly increased compared with the parental HCT116-*BUB1*$^{+/+}$ cells (losses in 26.3% of the metaphases; Figure 1*B*). These results support a causal relationship between *BUB1* haploinsufficiency and the occurrence of variegated aneuploidy.

[*]Authors share co-first authorship.

Abbreviations used in this paper: CRC, colorectal cancer; MVA, mosaic variegated aneuploidy; SAC, spindle assembly checkpoint.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2013.06.001

Table 1. Variants Identified in the Coding Regions of *BUB1* and *BUB3* and Clinical Information of Early-Onset CRC Patients

Patient no.	Ancestry	Gene[a]	Mutation[b]	Change	PolyPhen[c]	SIFT[c]	HOPE[c]	Tumor types (age, *y*)	Familial history of cancer[d]	Patient deceased (age, *y*)	Mosaic aneuploidy	Dysmorphic features[e]
1	European	BUB1	1.7 Mb deletion	Allelic loss	—	—	—	CRC (37)	No	No	Yes	Yes
2	Han Chinese	BUB1	c.46C>T	p.Gln16*	—	—	—	Jejunum (34); CRC (2X; 40); RCC (44); LC (2X; 45)	EC, 70; CRC, 42; Pol, 49	Yes (47)	ND	ND
3	Han Chinese	BUB1	c.2844T>	p.Gln949fs	—	—	—	CRC (31)	EC, 60; GC, 46; Pol, ?	Yes (36)	ND	ND
4	European	BUB3	c.790T>C	p.Phe264Leu	Possibly damaging	Deleterious	Possibly damaging	CRC (29); LC (44)	OC, 61	No	Yes	Yes
5	European	BUB3	c.63G>C	p.Lys21Asn	Possibly damaging	Deleterious	Possibly damaging	CRC (32)	HD, 32; Pol, 36	No	Yes	No
6	European	BUB3	c.446G>A	p.Arg149Gln	Benign	Tolerated	Possibly damaging	CRC (38); Pol (4X; 59–61)	LC, 78; BC, 68	Yes (71)	ND	ND

BC, breast cancer; EC, esophageal cancer; GC, gastric cancer; HD, Hodgkin's lymphoma; HOPE, have your protein explained; LC, lung cancer; ND, not determined; OV, ovarian cancer; Pol, colonic polyps; RCC, renal pelvis carcinoma SIFT, sorts intolerant from tolerant.

[a]National Center for Biotechnology Information Reference Sequence NM_004336.3 (*BUB1*) and NM_004725.3 (*BUB3*).

[b]Selection of variants is based on high interspecies conservation, predicted pathogenicity, and the unique occurrence of the variants identified.

[c]In silico prediction of impact of an amino acid substitution on the structure and function of a protein (see Supplementary Material).

[d]Tumors diagnosed in family member at indicated age.

[e]A full description of the patients and all dysmorphic features identified is described in the Supplementary Material.

Subsequent whole-exome sequencing of 10 Dutch and 23 Chinese patients with early-onset CRC revealed 3 additional mutations: a nonsense substitution in *BUB1* (patient 2, age 34 years, p.Gln16*), a 1-bp deletion in *BUB1* (patient 3, age 31 years, p.Gln949fs), both predicted to result in nonsense-mediated decay, and a highly conserved nonsynonymous missense mutation in another SAC gene, *BUB3* (patient 4, age 29 years, p.Phe264Leu; Figure 1*C* and *D*). Subsequently, we performed targeted copy number and mutation screening of a replication cohort of 146 familial or early-onset CRC patients, and 28 unsequenced samples from the initial cohort (n = 39) and identified 2 missense variants in *BUB3* that were absent in 1154 controls and were predicted to be pathogenic. One of these variants (patient 5, age 32 years, p.Lys21Asn) was found to be located in the core of the protein, and the second variant (patient 6, age 38 years, p.Arg149Gln) was predicted to destabilize one of the protein-interacting domains (Figures 1*C*–*F*). Next, we performed cytogenetic analyses of lymphocytes and primary skin fibroblasts of patients 4 and 5 (Supplementary Figure 2*A*). Patient 4 revealed variegated aneuploidies and structural abnormalities in 27% of the lymphocytes, and a mosaic (50%) trisomy 8 in fibroblasts, which was absent in lymphocytes, normal colon, and tumor tissue (data not shown). In patient 5, variegated aneuploidies and structural abnormalities were observed in 23% and 6.6% of lymphocytes and fibroblasts, respectively. In contrast, on average, only 5% of the lymphocytes from 10 healthy controls revealed aneuploidy, without chromosome gains or structural rearrangements (Supplementary Figure 2*B*). In addition, 2 mutation carriers (patients 1 and 2) presented with anomalies reminiscent of MVA syndrome (Table 1). Familial histories for cancer revealed that 8 of 12 parents of the patients were diagnosed with cancer (full description in the Supplementary Material and Supplementary Figure 5*A*).

Our data suggest that mutations or haploinsufficiency of the SAC genes *BUB1* and *BUB3* increase the risk of MVA developing in multiple tissues and CRC at a young age. The association between haploinsufficiency of SAC genes, MVA, and cancer predisposition is supported by studies in mice and humans.[3,9,12] The high mitotic rate of cells in the gastrointestinal tract can give rise to an increased cancer risk, but SAC gene defects can also increase the risk to develop other cancer types, such as lung cancer (patients 2 and 4). One of the *BUB1* mutation carriers (patient 2) also carries a pathogenic germline *MLH1* mutation, which is associated with Lynch syndrome, and all 4 of his tumors showed loss of both MLH1 and PMS2 expression. Segregation analysis in 3 siblings of patient 2 (Supplementary Figure 5*B*) led to the identification of an *MLH1* mutation-positive, *BUB1* wild-type sister who, at the age of 58 years, had not developed cancer. We, therefore, hypothesize that *BUB1* haploinsufficiency might have contributed to the development of multiple tumors in patient 2 by increasing the risk of losing the wild-type *MLH1* allele, as has been shown in mouse models.[5] *BUB1* might have acted as a

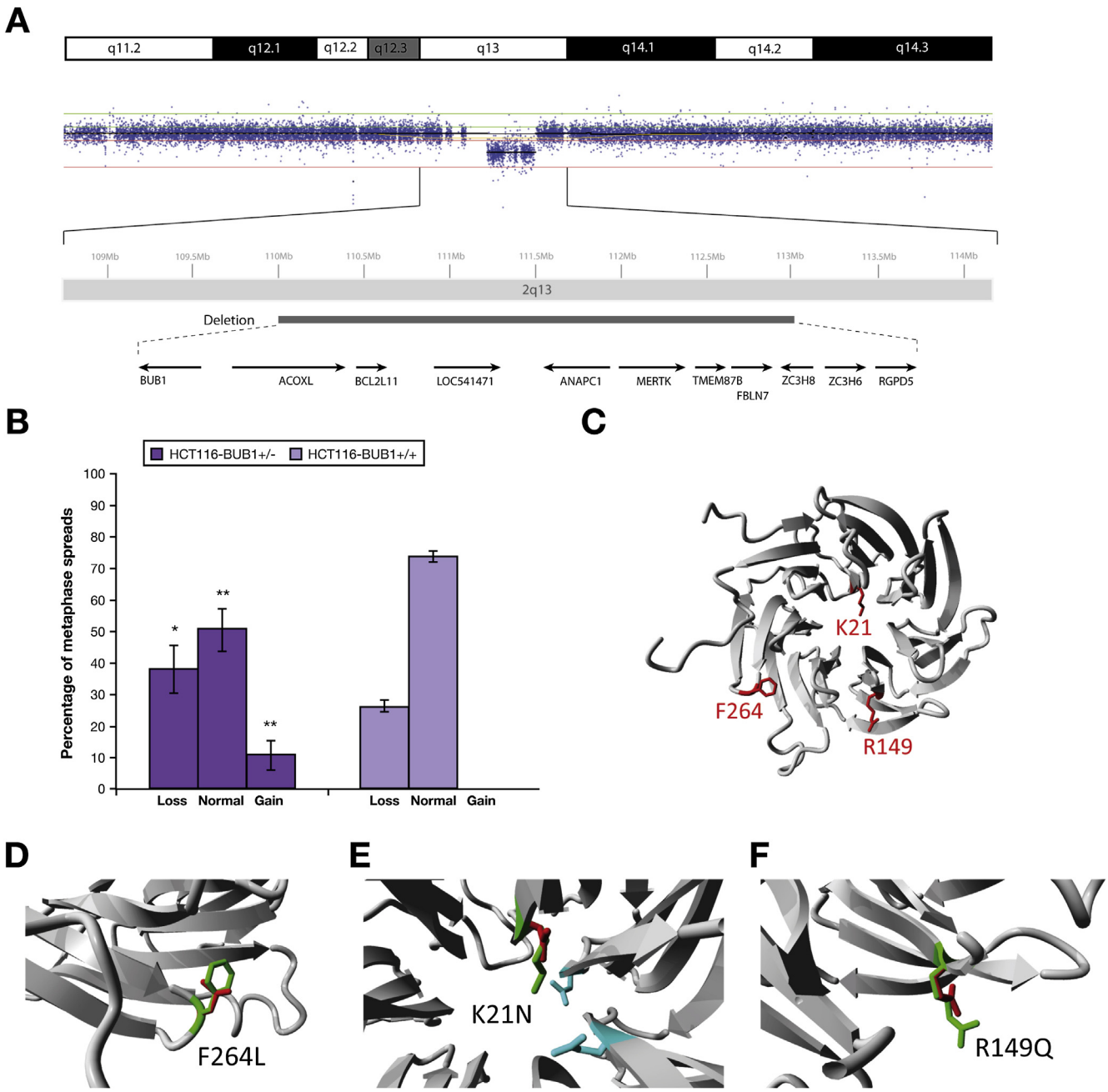

Figure 1. Identification of constitutional variants in *BUB1* and *BUB3*. (*A*) An approximately 1.75-Mb deletion affecting *BUB1* in patient 1. (*B*) Monoallelic disruption of *BUB1* in HCT116 cells causes chromosomal segregation defects. The percentages of cells with a chromosome loss, gain, or normal chromosome number in parental HCT116-*BUB1*$^{+/+}$ (n = 129) and haploinsufficient HCT116-*BUB1*$^{+/-}$ (n = 134) cells are shown. Haploinsufficient HCT116-*BUB1*$^{+/-}$ cells exhibit a significantly higher percentage of aneuploidies than parental HCT116-*BUB1*$^{+/+}$ cells. Error bars represent SD values; $^{*}P < .05$; $^{**}P < .01$. (*C*) Localization of the variants (*red*) identified in BUB3. (*D*) Close-up of residue 264: the replacement of a phenylalanine (*green*) by a leucine (*red*) can cause steric hindrance, thereby disturbing hydrophobic interactions. (*E*) Close-up of residue 21: a lysine (*green*) is replaced by an asparagine (*red*) and predicted to cause loss of interaction of residues (*blue*) in adjacent WD40 domains. (*F*) Close-up of residue 149: an arginine (*green*) is replaced by a glutamine (*red*). The predicted local conformational changes can affect interactions with other proteins.

modifier gene in CRC development in this patient. Unfortunately, most parents of the *BUB1* or *BUB3* mutation carriers were deceased and 2 families were no longer available for performing segregation analysis. Therefore, the identification of new families with *BUB1* or *BUB3* germline mutations in larger cohorts of CRC patients will be necessary to accurately estimate the lifetime cancer risk of these carriers.

In summary, we report a novel class of mutations that is associated with an increased risk to develop CRC. Next to the previously reported SAC component *BUB1B*, we show that heterozygous germline mutations in *BUB1* or *BUB3* recurrently occur in early-onset CRC cases. These cases present with a heterogeneous clinical phenotype reminiscent of MVA syndrome.[7,8] Additional delineation of these features will provide a basis for improved

recognition of individuals with SAC component anomalies and targeted prevention of primary and secondary cancers in carriers of such mutations.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2013.06.001.

References

1. Holland AJ, et al. Nat Rev Mol Cell Biol 2009;7:478–487.
2. Nicholson JM, et al. Adv Cancer Res 2011;112:43–75.
3. Babu JR, et al. J Cell Biol 2003;3:341–353.
4. Sotillo R, et al. Cancer Cell 2007;1:9–23.
5. Baker DJ, et al. Cancer Cell 2009;6:475–486.
6. Ricke RM, et al. J Cell Biol 2011;6:1049–1064.
7. García-Castillo H, et al. Am J Med Genet 2008;146A-13:1687–1695.
8. Hanks S, et al. Nat Genet 2004;11:1159–1161.
9. Rio Frio T, et al. N Engl J Med 2010;27:2628–2637.
10. de Voer RM, et al. N Engl J Med 2011;13:1279–1280.
11. Hanks S, et al. Cell Cycle 2005;2:225–227.
12. Baker DJ, et al. Curr Opin Cell Biol 2005;6:583–589.
13. Yu HE, et al. Clin Genet 2012;81:257–264.

Received January 1, 2013. Accepted June 1, 2013.

Reprint requests

Address requests for reprints to: Jian Q. Sheng, MD, Department of Gastroenterology, General Hospital of Beijing Military Region, Beijing 100700, China. e-mail: jianqiu@263.net; fax: +86-10-66721299; or Roland P. Kuiper, PhD, Department of Human Genetics 855, Radboud University Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands. e-mail: r.kuiper@gen.umcn.nl; fax: +31-24-3668752.

Acknowledgments

The authors thank the patients for their cooperation and Ineke Wienen, Bregt Roerdinkholder, Jorien Sanderink, Erik Jansen, Dr Birgit Sikkema-Raddatz, Dr Nicole de Leeuw and the Laboratory for Tumor Genetics for expert technical assistance, Drs Shi Rong Li, Ying Han, and Peng Jin for patient collections, and Dr N. Adachi (Yokohama City University) for providing the hygromycin-entry clone and pDEST DTA-MLS vectors. We thank Juan Tian and Zhimin Feng from BGI for providing Han Chinese population-specific mutation information and Prof Dr Han Brunner for critical reading of the manuscript.

Conflicts of interest

The authors disclose no conflicts.

Funding

This work was supported by research grants from the Dutch Cancer Society (KWF), the Netherlands Organization for Scientific Research (NWO), the Royal Dutch Academy of Sciences (KNAW), and the German Cancer Aid (Deutsche Krebshilfe).