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### **Citation**

Elsayed, F. A., Grolleman, J. E., Ragnathan, A., Buchanan, D. D., Wezel, T. van, & Voer, R. M. de. (2020). Monoallelic NTHL1 loss-of-function variants and risk of polyposis and colorectal cancer. *Gastroenterology*, 159(6), 2241-2243.e6.  
doi:10.1053/j.gastro.2020.08.042

Version: Publisher's Version  
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**Note:** To cite this publication please use the final published version (if applicable).



# Monoallelic *NTHL1* Loss-of-Function Variants and Risk of Polyposis and Colorectal Cancer

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**Keywords:** Colorectal Cancer; Base Excision Repair; Tumor Mutational Signatures; Mutation Carrier.

The endonuclease III-like protein 1, encoded by *NTHL1*, is a bifunctional glycosylase involved in base-excision repair (BER) that recognizes and removes oxidized pyrimidines.<sup>1</sup> Similar to biallelic loss-of-function (LoF) variants in *MUTYH*,<sup>2</sup> biallelic LoF variants in *NTHL1* predispose to colorectal polyps and colorectal cancer (CRC).<sup>3</sup> Recently, a multitumor phenotype was observed in individuals diagnosed with *NTHL1* deficiency.<sup>4</sup> Carriers of monoallelic pathogenic variants in *MUTYH* have an increased, albeit small, risk of CRC.<sup>5</sup> Thus far, it is unknown if monoallelic *NTHL1* LoF variants also increase the risk of polyposis and/or CRC. This information is especially important for carriers of the most common LoF variant in *NTHL1* (p.(Gln90\*); NM\_002528.5), which is heterozygous in approximately 0.28% of the general population.<sup>6</sup> Identification of monoallelic *NTHL1* LoF variants currently presents a clinical conundrum regarding how best to counsel carriers with respect to their cancer risk because of the lack of published evidence. Here, we show that monoallelic LoF variants in *NTHL1* are not enriched in individuals with polyposis and/or CRC compared to the general population. Furthermore, 13 colorectal tumors from *NTHL1* LoF carriers did not show a somatic second hit, and we did not find evidence of a main contribution of mutational signature SBS30, the signature associated with *NTHL1* deficiency, suggesting that monoallelic loss of *NTHL1* does not substantially contribute to colorectal tumor development.

## Methods

A total of 5,942 individuals with unexplained polyposis, familial CRC, or sporadic CRC at young age or suspected of having Lynch syndrome with CRC or multiple adenomas were included in this study and defined as case patients (individual studies and their ascertainment are described in [Supplementary Methods](#) and [Supplementary Table 1](#)). Three independent data sets were used as controls, including (1) the non-Finnish European subpopulation of the genome aggregation database (gnomAD: n = 64,328),<sup>6</sup> (2) a Dutch cohort of individuals without a suspicion of hereditary cancer who underwent whole-exome sequencing (WES) (Dutch WES; n = 2,329),<sup>7</sup> and (3) a population-based and cancer-affected

cohort from the Colon Cancer Family Registry Cohort (CCFRC; n = 1,207) ([Supplementary Methods](#) and [Supplementary Table 1](#)).

Pathogenic *NTHL1* LoF variants were identified in case patients by sequencing the exonic regions of *NTHL1* (n = 3,439) or by genotyping of 2 LoF variants in *NTHL1* (c.268C>T, p.(Gln90\*); n = 2503 and c.806G>A, p.(Trp269\*); n = 261) ([Supplementary Table 1](#)). For control individuals, all pathogenic LoF variants were retrieved from gnomAD and the Dutch WES-cohort,<sup>6,7</sup> and for the CCFRC control individuals, the exonic regions of *NTHL1* were sequenced ([Supplementary Table 1](#)). Odds ratios between case patients and control groups were calculated and a Fisher exact test was performed to assess the significance of difference in carrier rates. Cosegregation analysis was performed by using Sanger sequencing. Two adenomas and 11 primary CRCs from *NTHL1* LoF variant carriers were subjected to WES, and subsequently, mutational signature analysis was performed ([Supplementary Methods](#) and [Supplementary Table 2](#)). For signature analysis comparison, we included 3 CRCs from individuals with a biallelic *NTHL1* LoF variant.

## Results

Monoallelic *NTHL1* LoF variants were identified in 11 of 3,439 case patients (0.32%) and in 5 of 1,207 (0.41%) of CCFRC control individuals, indicating no significant difference ( $P = .784$ ) ([Figure 1A](#), [Supplementary Table 1](#)). Genotyping of the *NTHL1* p.(Gln90\*) variant in another 2,503 case patients identified 7 additional carriers (0.28%). The overall frequency of *NTHL1* p.(Gln90\*) in case patients was not different from the frequency in the gnomAD (17/5,942 vs 250/64,328;  $P = .914$ ), CCFRC (17/5,942 vs 3/1,207;  $P = .556$ ) or Dutch WES control individuals

\*Authors share co-first authorship; §Authors share co-senior authorship.

**Abbreviations used in this paper:** CCFRC, Colon Cancer Family Registry Cohort; CRC, colorectal cancer; LoF, loss of function; WES, whole-exome sequencing.

Most current article

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0016-5085

<https://doi.org/10.1053/j.gastro.2020.08.042>



insufficient to result in the accumulation of somatic mutations that are characteristic of an *NTHL1*-deficiency phenotype.

## Discussion

In this study, the largest investigating monoallelic LoF variants in *NTHL1* to date to our knowledge, we observed no evidence of an association between carriers and the risk of polyposis and/or CRC. In our case patients, the prevalence of pathogenic *NTHL1* LoF variant alleles is comparable to that of the general population. However, we cannot rule out that a small risk for CRC, similar to what is observed for *MUTYH* carriers, still exists.

Colorectal tumors from monoallelic *NTHL1* LoF variant carriers did not show evidence of a somatic second hit in *NTHL1* nor of defective base-excision repair, which is typically associated with biallelic *NTHL1* inactivation. Only 1 tumor showed a minor SBS30 contribution to the mutation profile, but this contribution was far less significant compared to *NTHL1*-deficient CRC and is likely the result of multiple testing correction. Our data suggest that inactivation of the *NTHL1* wild-type allele is a rare event in colorectal tumors, which is in agreement with the observation that loss of heterozygosity of chromosome arm 16p is not frequently observed in CRC.<sup>8</sup> We were unable to discriminate between individuals with polyposis or CRC due to the historical nature of the case collections. Therefore, differences in the frequencies of monoallelic *NTHL1* LoF variants between control individuals and these 2 phenotypes were not made separately. However, because we identified *NTHL1* LoF variants in individuals with polyposis or CRC, we do not consider a major difference between these 2 phenotypes. Because *NTHL1* deficiency may also predispose to extracolonic tumors, the risk for these tumor types in monoallelic *NTHL1* carriers still needs further assessment.

In conclusion, the evidence to date does not support an increased risk of polyposis and/or CRC for carriers of monoallelic *NTHL1* LoF variants, and consequently, no additional surveillance is currently warranted beyond population screening for CRC, unless family history characteristics point to a reason for colonoscopy.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at [www.cghjournal.org](http://www.cghjournal.org), and at <https://doi.org/10.1053/j.gastro.2020.08.042>.

## References

1. Krokan HE, Bjørås M. *Cold Spring Harb Perspect Biol* 2013;5:a012583.
2. Al-Tassan N, et al. *Nat Genet* 2002;30:227–232.
3. Weren RD, et al. *Nat Genet* 2015;47:668–671.

4. Grolleman JE, de Voer RM, Elsayed FA, et al. *Cancer Cell* 2019;35:256–266.
5. Win AK, et al. *Int J Cancer* 2011;129:2256–2262.
6. Karczewski KJ, et al. *Nature* 2020;581:434–443.
7. de Voer RM, Hahn MM, Mensenkamp AR, et al. *Sci Rep* 2015;5:14060.
8. Cerami E, et al. *Cancer Discov* 2012;2:401–404.

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Received June 17, 2020. Accepted August 22, 2020.

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### Acknowledgments

The authors thank all study participants, the CCFRC and staff, and the Dutch Parelstoer Institute Biobank Hereditary Colorectal Cancer for their contributions to this project. Furthermore, we would like to thank Robbert Weren, Eveline Kamping, M. Elisa Vink-Börger, Riki Willems, Christian Gillissen, Peggy Manders, Dina Ruano, Ruud van der Breggen, Marina Ventayol, Sanne ten Broeke, Allyson Templeton, Maggie Angelakos, members of the Colorectal Oncogenomics Group, Sharelle Joseland, Susan Preston, Julia Como, Thomas Green, Magda Kloc, and Chris Cotsopoulos for their contributions to this project. The author(s) would further like to acknowledge networking support by the Cooperation in Science and Technology Action CA17118, supported by the European Cooperation in Science and Technology.

*NTHL1* study group: Arnoud Boot, Marija Staninova Stojovska, Khalid Mahmood, Mark Clendenning, Noel de Miranda, Dagmara Dymerska, Demi van Egmond, Steven Gallinger, Peter Georgeson, Nicoline Hoogerbrugge, John L. Hopper, Erik A.M. Jansen, Mark A. Jenkins, Jihoon E. Joo, Roland P. Kuiper, Marjolijn J.L. Ligtenberg, Jan Lubinski, Finlay A. Macrae, Hans Morreau, Polly Newcomb, Maartje Nielsen, Claire Palles, Daniel J. Park, Bernard J. Pope, Christophe Rosty, Clara Ruiz Ponte, Hans K. Schackert, Rolf H. Sijmons, Ian P. Tomlinson, Carl M. J. Tops, Lilian Vreede, Romy Walker, Aung K. Win, Colon Cancer Family Registry Cohort Investigators, Aleksandar J. Dimovski, and Ingrid M. Winship.

### CRedit Authorship Contributions

Fadwa A. Elsayed, MSc (Data curation: Equal; Formal analysis: Equal; Writing – original draft: Equal); Judith E. Grolleman, MSc (Data curation: Equal; Formal analysis: Equal; Visualization: Equal; Writing – original draft: Equal); Abiram Ragunathan, MBBS (Data curation: Equal; Formal analysis: Equal; Visualization: Equal; Writing – original draft: Equal); Daniel D. Buchanan, PhD (Conceptualization: Equal; Formal analysis: Equal; Funding acquisition: Equal; Supervision: Equal; Writing – original draft: Equal; Writing – review & editing: Equal); Tom van Wezel, PhD (Conceptualization: Equal; Formal analysis: Equal; Funding acquisition: Equal; Supervision: Equal; Writing – review & editing: Equal); Richarda M. de Voer, PhD (Conceptualization: Equal; Formal analysis: Equal; Funding acquisition: Equal; Supervision: Equal; Writing – original draft: Equal; Writing – review & editing: Equal).

### Conflicts of interest

The authors disclose no conflicts.

### Funding

This study was funded by research grants from the Dutch Cancer Society (KUN2015-7740), the Dutch Digestive Foundation (MLDS FP13-13 to Tom van Wezel), Instituto de Salud Carlos III and European Regional Development Fund (ERDF) (PI14/00230 to Clara Ruiz Ponte) and by grant UM1 CA167551 from the National Cancer Institute and through cooperative agreements with the following Colon Cancer Family Registry Cohort (CCFRC) sites: Australasian Colorectal Cancer Family Registry (U01 CA074778 and U01/U24 CA097735), Ontario Familial Colorectal Cancer Registry (U01/U24 CA074783), and Seattle Colorectal Cancer Family Registry (U01/U24 CA074794). Daniel B. Buchanan is a University of Melbourne Research at the Melbourne Accelerator Program (R@MAP), principal research fellow, and National Health and Medical Research Council (NHMRC) R.D. Wright Career Development Fellow. Abiram Ragunathan is a Melbourne Genomics Health Alliance Fellow.

## Supplementary Methods

### Study Cohorts

We included 5,942 patients with unexplained polyposis, familial CRC, or sporadic CRC at a young age or suspected of having Lynch syndrome with CRC or multiple adenomas (Supplementary Table 1) from the Netherlands (n = 3,158); United Kingdom (n = 275); Poland (n = 144); Germany (n = 104); Spain (n = 35); North Macedonia (n = 273); and North America, Canada, and Australia (CCFRC; n = 1,953).<sup>1-3</sup> All participants provided written informed consent. Local medical ethical committees approved this study (Radboudumc [Commissie mensgebonden onderzoek (CMO)-light, 2015/2172 and 2015/1748], Leiden University Medical Center [LUMC] [P01-019], and Ontario Cancer Research Ethics Board, University of Melbourne Human Research Ethics Committee, and Fred Hutchinson Cancer Research Center institutional review board).

A total of 1,207 cancer-unaffected control individuals were available from the population-based recruitment arms of the CCFRC.<sup>2,3</sup> From the Netherlands, 2,329 WES control individuals with a >90-fold median coverage without a suspicion of hereditary cancer were available.<sup>4</sup> The European non-Finnish population of gnomAD was used to determine overall frequencies of LoF variants.<sup>5</sup>

### Targeted Resequencing

**Hi-Plex.** Leukocyte DNA from 1,953 CRC-affected case patients and 1,207 control individuals was used to screen the coding regions of *NTHL1* by using multiplex polymerase chain reaction (PCR)-based targeted sequencing and variant calling approach (HiPlex2 and Hiplexpipe, [hiplex.org](http://hiplex.org), [github.com/khalidm/hiplexpipe](https://github.com/khalidm/hiplexpipe)).<sup>6</sup> Germline variants in *NTHL1* (NM\_002528.5) were prioritized according to quality—the sequence depth of >30 reads and variant frequency of >30%.

**Molecular Inversion Probe-Based Sequencing.** Leukocyte DNA from 1,486 polyposis and/or CRC cases was screened for all coding regions and intron-exon boundaries of *NTHL1* (NM\_002528.5) by using molecular inversion probe MIPsequencing, combined with a panel of base excision repair genes, as described previously.<sup>1</sup> Reads were mapped with Burrows-Wheeler Aligner (BWA), and variant calling was performed with UnifiedGenotyper.<sup>7</sup> Somatic variants in *NTHL1* were prioritized according to quality: sequence depth of >40 reads, >20 variant reads, variant frequency of >25%, and quality by depth scores >8,000.

Variants from HiPlex and MIP screenings were further selected based on predicted LoF of *NTHL1*. We selected all nonsense, frameshift canonical splice sites and included only coding and noncoding splice site region variants with a predicted change of >20%, based on Alamut (Interactive Biosoftware, Rouen, France) (MaxEnt, NNSplice, and Human Splicesite Finder [HSF]).

### KASPar Assay

Leukocyte DNA (n = 1,260) or germline DNA extracted from formalin-fixed, paraffin embedded (FFPE) surgical

specimens (n = 982) was genotyped for *NTHL1* p.(Gln90\*) by using KBioscience Competitive Allele-Specific PCR (KASPar) assay.<sup>1</sup>

### Allele-Specific Polymerase Chain Reaction

Leukocyte DNA from 261 individuals with sporadic or familial CRC was subjected to an allele-specific PCR (AS-PCR) specific for *NTHL1* p.(Gln90\*) and p.(Trp269\*); primers are available upon request.

### Sanger Sequencing

Sanger sequencing was used for variant validation and to sequence the entire open reading frame of *NTHL1* in confirmed heterozygous cases. In addition, when available, family members were sequenced by using Sanger sequencing for cosegregation purposes.

### Statistical analysis

A 1-sided Fisher exact test was performed to determine differences in the frequency of monoallelic *NTHL1* germline LoF variants in carriers with polyposis and/or CRC compared to control individuals. We calculated the *P* value, odds ratio, and the 95% confidence interval using R (R Foundation for Statistical Computing, Vienna, Austria; <http://www.R-project.org>). Three control data sets were used in this comparison.

First, we retrieved all LoF variants (nonsense, frameshift canonical splice sites, and coding or noncoding splice site regions with >20% splice site change) in canonical transcripts of *NTHL1* listed in the non-Finnish European subpopulation of the genome aggregation database (gnomAD).<sup>5</sup> All variants were checked manually in gnomAD for their quality. Second, LoF variants in *NTHL1* identified in the Dutch WES cohort (n = 2,329 individuals without a suspicion of hereditary cancer) were extracted in a similar way as described earlier.<sup>4</sup> Third, LoF variants in *NTHL1* identified in the CCFRC control group of 1,207 individuals, sequenced in this study, were used.

### Whole-Exome Sequencing

Exome captures (Supplementary Table 2) were performed according to the manufacturer by using either Agilent Clinical Research Exome (CRE) V2 (Agilent, Santa Clara, CA) in combination with sequencing on a NovaSeq 6000 (Illumina, San Diego, CA), Agilent SureSelect XT<sup>HS</sup> Human All Exon V6 enrichment kit in combination with sequencing on a NextSeq 500, or xGEN Exome Research Panel (Integrated DNA Technology [IDT], Coralville, IA) in combination with sequencing on a NovaSeq 6000.

Novaseq 6000 sequencing reads were trimmed by using Trimmomaticv0.36 and aligned to hs37d5 by using BWA-MEM, followed by merging and PCR duplicate removal with Sambamba (version 0.5.8).<sup>8,9</sup> Variant calling was performed by using Strelka (version 2.017) and Freebayes for paired samples; only variants called by both callers were reported.<sup>10,11</sup> For LUMC2745, no paired sample was available, and variant calling was performed with Mutect2 (GATK version 4.1.0.0; GATK, Broadinstitute, Cambridge, MA).

Trimmed NextSeq 500 sequencing reads were aligned to GRCh37 by using BWA-MEM, and duplicates were flagged by using Picard Tools, version 1.90. Variants were called with Mutect2 (GATK version 4.1.0.0), with or without matched germline samples; variant filtering was performed as described,<sup>1</sup> with minor modifications. Variants in dbSNPv132 (minus catalogue of somatic mutations in cancer [COSMIC]), microsatellites, homopolymers, simple repeats, and variants called outside of the respective exome capture target were removed. Somatic variants with a variant allele frequency of <10%, <20× coverage in both normal and tumor, and fewer than 4 reads supporting the variant were removed. For tumor-only analysis, variants shared by more than 1 individual and variants with a variant allele frequency of >80% were removed to reduce germline leakage.

### Mutational Signature Analysis

Mutation spectra were generated by using In-depth characterization and analysis of mutational signatures (ICAMS), version 2.1.2 (github.com/steverozen/ICAMS), and mutational signature analysis was performed by using mSigAct v2.0.0.9018.<sup>12</sup> Tissue-specific CRC signature universes were inferred from the Pan-cancer analysis of whole genomes (PCAWG) signature assignments.<sup>13</sup> The signature universe was extended with SBS30 and potential artefact signatures SBS45, SBS51, SBS52, SBS54, and SBS58, which were present in a subset of the samples of this cohort. Signatures were normalized to the trinucleotide abundance of the respective exome capture panel used. Per mutation spectrum, mutational signature assignment was performed by using mSigAct::SparseAssignActivity, with  $P = .5$  to reduce sparsity. The presence of SBS30 was then determined using mSigAct::SignaturePresenceTest using the signatures determined by mSigAct::SparseAssignActivity plus SBS30 as well as the aging-associated signatures SBS1, SBS5, and SBS40 (Supplementary Table 2). Multiple testing correction was done according to Benjamini-Hochberg.

## References

1. **Grolleman JE, de Voer RM, Elsayed FA, et al.** Mutational signature analysis reveals NTHL1 deficiency to cause a multi-tumor phenotype. *Cancer Cell* 2019; 35:256–266.
2. Jenkins MA, Win AK, Templeton AS, et al. Cohort Profile: The Colon Cancer Family Registry Cohort (CCFRC). *Int J Epidemiol* 2018;47:387–388i.
3. Newcomb PA, Baron J, Cotterchio M, et al. Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:2331–2343.
4. **de Voer RM, Hahn MM, Mensenkamp AR, et al.** Deleterious germline BLM mutations and the risk for early-onset colorectal cancer. *Sci Rep* 2015;5:14060.
5. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2020;581:434–443.
6. Hammet F, Mahmood K, Green TR, et al. Hi-Plex2: a simple and robust approach to targeted sequencing-based genetic screening. *Biotechniques* 2019;67:118–122.
7. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;43:491–498.
8. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
9. Tarasov A, Vilella AJ, Cuppen E, et al. Sambamba: fast processing of NGS alignment formats. *Bioinformatics* 2015;31:2032–2034.
10. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. arXiv; 2012. Available at: <https://arxiv.org/abs/1207.3907v2> Accessed October 25, 2020.
11. Saunders CT, Wong WS, Swamy S, et al. Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics* 2012;28:1811–1817.
12. **Ng AWT, Poon SL, Huang MN, et al.** Aristolochic acids and their derivatives are widely implicated in liver cancers in Taiwan and throughout Asia. *Sci Transl Med* 2017;9(412):eaan6446.
13. **Alexandrov LB, Kim J, Haradhvala NJ, et al.** The repertoire of mutational signatures in human cancer. *Nature* 2020;578:94–101.
14. Vos JR Manders P, de Voer RM, et al. Parelsoer Institute Biobank Hereditary Colorectal Cancer: a joint infrastructure for patient data and biomaterial on hereditary colorectal cancer in the Netherlands. *Open J Bioresources* 2019;6;1; Doi: <http://doi.org/10.5334/ojb.54>.

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**Supplementary Table 1.** Characteristics of Case and Control Cohorts and Identified Case Patients and Control Individuals With Monoallelic *NTHL1* LoF Variants in This Study

Approach	Sequencing method and cohorts	Samples, n	Selection <sup>a</sup> criteria	Genes tested	Monoallelic <i>NTHL1</i> p.(Gln90*), n	Other monoallelic <i>NTHL1</i> LoF variants, n	Total monoallelic <i>NTHL1</i> LoF variants, n
<i>NTHL1</i> -targeted resequencing (n = 3,439 cases)	Hi-Plex multiplex PCR-based sequence screening of <i>NTHL1</i> exons (control individuals)						
	Colon Cancer Family Registry	1,207	Population-based healthy individuals with no history of polyposis and/or CRC	NA	3	2	5
	Hi-Plex multiplex PCR based sequence screening of <i>NTHL1</i> exons (case patient)						
	Colon Cancer Family Registry	1,953	Population-based CRC	<i>APC, MUTYH, POLE, POLD1, MMR*</i>	4	1	5
	MIP-based sequence screening of <i>NTHL1</i> (case patients)						
	ParelBED (the Netherlands <sup>b</sup> )	600	Polyposis, CRC, or CRC and additional tumor	No disease-causing mutation found after routine diagnostics	0	0	0
	Oxford (United Kingdom)	275	Polyposis	<i>APC, MUTYH</i>	4	0	4
	Leiden (the Netherlands)	150	Polyposis or familial CRC	<i>APC, MUTYH</i>	0	0	0
	Nijmegen (the Netherlands)	147	Polyposis or familial CRC	<i>APC, MUTYH</i>	0	0	0
	Szczecin (Poland)	144	Familial CRC	<i>POLE, POLD1, MMR*<sup>b</sup></i>	1	0	1
	Dresden (Germany)	104	Polyposis or familial CRC	<i>APC, MUTYH</i>	0	0	0
	Santiago de Compostela (Spain)	35	Polyposis or familial CRC	<i>APC, MUTYH</i> (in part), <i>POLE, POLD1, BMPR1A, SMAD4, PTEN</i>	0	0	0
	Groningen (the Netherlands)	19	Polyposis or familial CRC	<i>APC, MUTYH</i>	0	0	0
Skopje (North Macedonia)	12	Polyposis, recessive inheritance	<i>MMR*<sup>b</sup>, APC, TP53, MUTYH, POLE, POLD1</i>	1	0	1	

Supplementary Table 1. Continued

Approach	Sequencing method and cohorts	Samples, n	Selection <sup>a</sup> criteria	Genes tested	Monoallelic NTHL1 p.(Gln90*), n	Other monoallelic NTHL1 LoF variants, n	Total monoallelic NTHL1 LoF variants, n
<i>NTHL1</i> genotyping (n = 2,503 cases)	<i>NTHL1</i> p.(Gln90*) genotyping by KASPar assay (case patients)						
	Leiden (the Netherlands)	1,894	Polyposis or familial CRC, with or without suspected Lynch syndrome	<i>APC</i> , <i>MUTYH</i> , <i>POLE</i> , <i>POLD1</i> , <i>MMR</i> <sup>b</sup>	3	NA	3
	Nijmegen (the Netherlands)	348	Polyposis or familial CRC	<i>APC</i> , <i>MUTYH</i> , <i>POLE</i> , <i>POLD1</i> , <i>MMR</i> <sup>b</sup>	1	NA	1
	<i>NTHL1</i> p.(Gln90*) and p.(Trp269*) genotyping by allele specific-PCR (case patients)						
	Skopje (North Macedonia)	200	Sporadic CRC	None	2	0	2
	Skopje (North Macedonia)	61	Polyposis or familial CRC	TruSight Hereditary Cancer Panel (Illumina)	1	0	1

NA, not applicable; ParelBED, The Dutch Parelnoer Institute Biobank Hereditary Colorectal Cancer.<sup>14</sup>

<sup>a</sup>Polyposis is defined as the cumulative occurrence of at least 10 polyps. Familial CRC is defined as the proband having a CRC  $\leq$ 50 years of age and at least 1 first degree relative with CRC  $\leq$ 60 years of age. Sporadic CRC is defined as patients with CRC without a family history, irrespective of age.

<sup>b</sup>MMR\* genes: *MLH1*, *MSH2*, *MSH6* and *PMS2*.

**Supplementary Table 2.** Phenotypic Description and Details on the Tumors Subjected to WES of Identified Carriers of a Monoallelic *NTHL1* LoF Variant

Number	Patient ID	Identification method	Amino acid change	Sex	Polyps	Malignancies <sup>f</sup>	Tumor type for WGS	Matched normal available	Exome enrichment kit	Sequencing platform	Median coverage tumor(s) <sup>g</sup>	Number of somatic variant calls	<i>P</i> value SBS30 <sup>a</sup>
1	P09708	Hi-Plex	p.(Gln287*)	M		Cecum (73), CRC (73)	CRC	Yes, blood	Agilent CRE V2	Novaseq 6000	221	572	.976
2	P92662	Hi-Plex	p.(Gln90*)	M		CRC (53)	CRC	Yes, blood	Agilent CRE V2	Novaseq 6000	189	219	1.61 × 10 <sup>-3</sup>
3	P07001	Hi-Plex	p.(Gln90*)	M		CRC (43)	CRC	Yes, blood	Agilent CRE V2	Novaseq 6000	116	141	.331
4	P58832	Hi-Plex	p.(Gln90*)	F		CRC (46), UC (29)	—	—	—	—	—	—	—
5	P00387	Hi-Plex	p.(Gln90*)	F		Cecum (42), UC (23), LC (53)	—	—	—	—	—	—	—
6	P0011 <sup>b</sup>	MIP screen	p.(Gln90*)	M		CRC (56), LiC (unk)	CRC	No <sup>c</sup>	Agilent V6	NextSeq500	133	1,466	.976
7	P0011-2 <sup>b</sup>	Cosegregation	p.(Gln90*)	F		CRC (55)	CRC	Yes, FFPE	Agilent V6	NextSeq500	86	292	.953
8	P0804	MIP screen	p.(Gln90*)	F		CRC (50)	CRC <sup>d</sup>	Yes, FFPE	Agilent V6	NextSeq500	—	—	—
9	P0468 <sup>e</sup>	MIP screen	p.(Gln90*)	M	A (43)		—	—	—	—	—	—	—
10	P0567 <sup>e</sup>	Co-segregation	p.(Gln90*)	F	A (55)		—	—	—	—	—	—	—
11	P0567-2 <sup>e</sup>	Co-segregation	p.(Gln90*)	F	A (61)		—	—	—	—	—	—	—
12	P0523	MIP screen	p.(Gln90*)	M	A (59)	CRC (58)	—	—	—	—	—	—	—
13	P0568	MIP screen	p.(Gln90*)	M	A (unk)		—	—	—	—	—	—	—
14	P0602	MIP screen	p.(Gln90*)	F	A (unk)		—	—	—	—	—	—	—
15	K134	KASPar assay	p.(Gln90*)	F	A (48-56)	CRC (49)	—	—	—	—	—	—	—
16	LUMC3333	KASPar assay	p.(Gln90*)	M		CRC (<69), Cecum (69)	CRC	Yes, FFPE	IDT xGEN	Novaseq 6000	131	150	.888
17	LUMC2745	KASPar assay	p.(Gln90*)	M		CRC (72); CRC, SCC (61)	CRC	No	IDT xGEN	Novaseq 6000	99	487	.053
18	LUMC0748	KASPar assay	p.(Gln90*)	F		CRC (56), OvC (56), CRC (56), CRC (68)	CRC	Yes, FFPE	IDT xGEN	Novaseq 6000	84	150	>.99
19	Tcc136	AS-PCR	p.(Gln90*)	M		CRC (75)	CRC <sup>f</sup>	No	Agilent V6	NextSeq500	195	192	.331

Supplementary Table 2. Continued

Number	Patient ID	Identification method	Amino acid change	Sex	Polyps	Malignancies <sup>i</sup>	Tumor type for WGS	Matched normal available	Exome enrichment kit	Sequencing platform	Median coverage tumor(s) <sup>j</sup>	Number of somatic variant calls	<i>P</i> value SBS30 <sup>a</sup>
20	Tcc456	AS-PCR	p.(Gln90*)	M		PC, CRC (72)	CRC	No	Agilent V6	NextSeq500	140	211	.052
21	Tcc712	AS-PCR	p.(Gln90*)	F	7A (71)	EC (66), CRC (71)	CRC <sup>f</sup>	No	Agilent V6	NextSeq500	180	4,083	1
22	P03-I:1	<sup>g</sup>	p.(Gln90*)	M		A, HP	A	No	IDT xGEN	Novaseq 6000	T1 = 64 T2 = 39	T1 = 81 T2 = 290	T1 = 1 T2 = .088
—	P04-II:5	<sup>g</sup>	p.Gln90*/ p.Ile245Asnfs*28	F	—	—	NTHL1-deficient CRC	Yes, FFPE	IDT xGEN	Novaseq 6000	162	347	3.11 × 10 <sup>-45</sup>
—	P05001	Hi-Plex	p.(Gln90*)/ p.(Ala79fs)	F	A, HP (61)	CRC (61), BCC (63)	NTHL1-deficient CRC	Yes, blood	Agilent CRE V2	Novaseq 6000	108	430	1.82 × 10 <sup>-39</sup>
—	CRC-3	<sup>h</sup>	p.(Gln90*)/ p.(Gln90*)	M	—	—	NTHL1-deficient CRC	Grolleman et al <sup>1</sup>	Grolleman et al <sup>1</sup>	Grolleman et al <sup>1</sup>	Grolleman et al <sup>1</sup>	360	3.08 × 10 <sup>-38</sup>

A, colorectal adenomatous polyps; BCC, basal cell carcinoma; EC, endometrial cancer; HP, hyperplastic polyps; ID, identifier; LC, lung cancer; LiC, liver cancer; OvC, ovarian cancer; PC, prostate cancer; SCC, squamous cell carcinoma; UC, uterine cancer; unk, age unknown; —, not applicable.

<sup>a</sup>Fresh-frozen tumor material.

<sup>b</sup>Sibling.

<sup>c</sup>The normal sample of the sibling was used for somatic variant extraction.

<sup>d</sup>Tumor P0804 was excluded from further analysis because of insufficient data quality.

<sup>e</sup>Sibling.

<sup>f</sup>Multiple testing correction was done according to Benjamini-Hochberg.

<sup>g</sup>Identified by Grolleman et al, 2019.<sup>1</sup>

<sup>h</sup>Tumor data from Grolleman et al, 2019.<sup>1</sup>

<sup>i</sup>Numbers in parentheses indicate age at diagnosis.

<sup>j</sup>Median read coverage (units = reads).