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Germline *TP53* Mutations in Patients With Early-Onset Colorectal Cancer in the Colon Cancer Family Registry

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Abstract

IMPORTANCE—Li-Fraumeni syndrome, usually characterized by germline *TP53* mutations, is associated with markedly elevated lifetime risks of multiple cancers, and has been linked to an increased risk of early-onset colorectal cancer.

OBJECTIVE—To examine the frequency of germline *TP53* alterations in patients with early-onset colorectal cancer.

DESIGN, SETTING, AND PARTICIPANTS—This was a multicenter cross-sectional cohort study of individuals recruited to the Colon Cancer Family Registry (CCFR) from 1998 through 2007 (genetic testing data updated as of January 2015). Both population-based and clinic-based patients in the United States, Canada, Australia, and New Zealand were recruited to the CCFR. Demographic information, clinical history, and family history data were obtained at enrollment. Biospecimens were collected from consenting probands and families, including microsatellite instability and DNA mismatch repair immunohistochemistry results. A total of a 510 individuals diagnosed as having colorectal cancer at age 40 years or younger and lacking a known hereditary cancer syndrome were identified from the CCFR as being potentially eligible. Fifty-three participants were excluded owing to subsequent identification of germline mutations in DNA mismatch repair genes (n = 47) or biallelic *MUTYH* mutations (n = 6).

INTERVENTIONS—Germline sequencing of the *TP53* gene was performed. Identified *TP53* alterations were assessed for pathogenicity using literature and international mutation database searches and *in silico* prediction models.

MAIN OUTCOMES AND MEASURES—Frequency of nonsynonymous germline *TP53* alterations.

RESULTS—Among 457 eligible participants (314, population-based; 143, clinic-based; median age at diagnosis, 36 years [range, 15–40 years]), 6 (1.3%; 95%CI, 0.5%–2.8%) carried germline missense *TP53* alterations, none of whom met clinical criteria for Li-Fraumeni syndrome. Four of the identified *TP53* alterations have been previously described in the literature in probands with clinical features of Li-Fraumeni syndrome, and 2 were novel alterations.

CONCLUSIONS AND RELEVANCE—In a large cohort of patients with early-onset colorectal cancer, germline *TP53* mutations were detected at a frequency comparable with the published prevalence of germline *APC* mutations in colorectal cancer. With the increasing use of multigene next-generation sequencing panels in hereditary cancer risk assessment, clinicians will be faced with the challenge of interpreting the biologic and clinical significance of germline *TP53* mutations in families whose phenotypes are atypical for Li-Fraumeni syndrome.

More than 10% of all colon cancers and nearly one-fifth of all rectal cancer diagnoses occur in patients younger than 50 years, yet the minority of early-onset cases can be attributed to the 3 most common hereditary colorectal cancer (CRC) syndromes: Lynch syndrome,

familial adenomatous polyposis (FAP), or *MUTYH*-associated polyposis (MAP).[1–4] Other hereditary syndromes linked to early onset CRC include Peutz-Jeghers syndrome, Cowden syndrome, juvenile polyposis, and Li-Fraumeni syndrome (LFS), yet their prevalence is poorly understood.[5,6]

Li-Fraumeni syndrome is an inherited cancer syndrome, usually caused by germline *TP53* mutations, in which patients classically develop early-onset cancers, including leukemias, brain tumors, sarcomas, breast carcinomas, and adrenocortical carcinomas.[7–10] Beyond these so-called core cancers, data have shown that *TP53* mutation carriers are also at increased risk for a wide array of other malignant neoplasms, including bronchoalveolar, pancreatic, gastric, ovarian, and colorectal cancers.[6,10–12] Germline *TP53* testing is recommended for individuals who meet strict clinical criteria, including classical LFS criteria[8] or Chompret criteria,[13,14] none of which include CRC as a component cancer.

With data linking germline *TP53* mutations to early-onset CRC, however, *TP53* testing is included on most multigene next-generation sequencing panels now commercially available for hereditary CRC risk assessment.[6,15] As the availability of such panels grows, the number of patients undergoing *TP53* mutation analysis will likely markedly increase.[15] In order for clinicians to provide effective and appropriate counseling to patients undergoing *TP53* testing as part of multigene risk assessment, an accurate understanding of this gene's contribution to hereditary and early-onset CRC is needed. This study's aim was to estimate the proportion of participants with early-onset CRC who carry germline *TP53* mutations.

Methods

Colon Cancer Family Registry

The Colon Cancer Family Registry (CCFR) is an international consortium created in 1997 to facilitate collaboration for interdisciplinary studies in the genetic epidemiology of CRC.[16] The CCFR consists of participants and families ascertained through both clinic-based and population-based recruitment in the United States, Canada, Australia, and New Zealand. All participants provided written informed consent for the use of blood samples and tumor tissue in cancer research and for inclusion in the CCFR through one of the following registry centers: Mayo Clinic (Rochester, Minnesota), Fred Hutchinson Cancer Research Center (Seattle, Washington), University of Southern California Consortium (Los Angeles), Lunenfeld Tanenbaum Research Institute (Toronto, Ontario, Canada), and University of Melbourne (Melbourne, Australia). Demographic information, clinical history, and family history data were obtained using standardized instruments, as previously described.[16] Biospecimens, including tumor tissue, were collected from consented probands and families, along with data on tumor microsatellite instability (MSI) and DNA mismatch repair (MMR) immunohistochemistry (IHC) status. All protocols were approved by the appropriate institutional review boards. All samples and data have been anonymized. Patients were not compensated for their participation.

Study Population

Participants enrolled in the CCFR were potentially eligible for analysis if they were diagnosed as having CRC at age 40 years or younger and were not known to carry a germline mutation in any of the genes linked to Lynch syndrome (*MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM*) or MAP. The CCFR has not routinely recruited individuals with FAP phenotypes and has not performed systematic germline *APC* analysis on enrolled participants. A few individuals with known germline *APC* mutations who had been enrolled at individual CCFR sites were included in the pool of potentially eligible participants for this study.

Germline Analysis

Archived genomic DNA from 510 potentially eligible participants was analyzed by a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Laboratory for Molecular Medicine, Cambridge, Massachusetts) using bidirectional Sanger sequencing of the 11 exons of the *TP53* gene (NM_000546) (see eMethods in the Supplement).

PubMed searches and querying of the International Agency for Research on Cancer *TP53* Mutation Database[17,18] were used to identify previous literature reports of the specific *TP53* alterations and assess their functional significance. Alamut mutation interpretation software (Interactive Biosoftware) was used to access PolyPhen-2 and SIFT for *in silico* pathogenicity analyses and to assess species conservation.[19,20] Minor allele frequencies (MAFs) for all identified alterations were queried from the Exome Variant Server.[21]

Results

Fifty-three participants were excluded from analysis owing to subsequent testing that identified pathogenic germline mutations in *MLH1* (n = 28 participants), *MSH2* (n = 14), *MSH6* (n = 2), *PMS2* (n = 3), and biallelic *MUTYH* mutations (n = 6). The final study population consisted of 457 participants with a history of CRC at age 40 years or younger (median age at diagnosis, 36 years [range, 15–40 years]). Microsatellite instability and/or MMR IHC results were available on tumors from 326 participants (71%), 47 of whom had MSI-H and/or mismatch repair deficient (MMR-D) findings (Table 1). A total of 162 participants (35%) had prior negative or inconclusive germline testing of at least 1 MMR gene (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). A total of 397 participants (87%) had prior *MUTYH* testing, 387 of whom had negative results, and 10 of whom carried monoallelic *MUTYH* mutations.

Six of the 457 participants (1.3%; 95%CI, 0.5%–2.8%) were found to carry germline *TP53* missense alterations that have not been previously described as benign changes. Four were recruited through population-based ascertainment, and 2 through clinic-based ascertainment. Statistical significance was set at $P = .05$. There was no significant difference in the clinical and pathological characteristics of *TP53* carriers vs non-carriers, except that 4 of the *TP53* carriers' CRCs were left-sided tumors ($P = .01$); the sites of the remaining 2 carriers' tumors were undefined. Microsatellite instability status was available for 2 of the carriers' tumors, both of which were microsatellite stable. Based on available clinical data, none of these 6

probands met either classic LFS criteria[8] or Chompret criteria[13,14] for germline *TP53* analysis. Two of the *TP53* probands were younger than 30 years at the time of CRC diagnosis. A Multiplex Ligation-dependent probe Amplification (MLPA) analysis of a randomly selected subset of 100 participants did not find any *TP53* deletions.

Participant 1 (of the 6 carrying the germline *TP53* missense alterations) was a white man with a prior melanoma at age 34 years who was diagnosed as having synchronous sigmoid adenocarcinomas at age 38 years (Figure), and carried a c.445C>T *TP53* alteration, resulting in the amino acid change p.Pro152Leu (Table 2). Germline *TP53* p.Pro152Leu alterations have been reported numerous times in the literature in probands meeting Chompret criteria,[14] including a proband with a pediatric choroid plexus carcinoma.[22] The p.Pro152Leu *TP53* alteration was also described in multiple reports of pediatric adrenocortical carcinoma probands, several of whom had relatives with histories of breast cancer and other malignant neoplasms, who carried the p.Pro152Leu alteration.[23–25] Our prior report[12] on gastric cancer in LFS included a family with the p.Pro152Leu *TP53* alteration, in which 3 separate carriers were diagnosed as having gastric carcinoma at ages 45, 52, and 58 years.

Participant 2 was a white man with sigmoid adenocarcinoma at age 37 years, who carried a c.1136G>A (p.Arg379His) *TP53* alteration. To our knowledge, germline p.Arg379His *TP53* alterations have not been previously described in the literature. A somatic p.Arg379His *TP53* alteration has been described in a patient with an astrocytoma; however, that patient's tumor had another somatic *TP53* alteration (p.Val218Gly)with a conservative amino acid change as well as 2 silent somatic *TP53* alterations, prompting the authors of the case report to label the p.Arg379His alteration "possibly noncausative."[26]

Participant 3 was a white man with CRC at age 33 years, who carried a c.869G>A (p.Arg290His) *TP53* alteration. Germline p.Arg290His *TP53* alterations were reported in a Portuguese family[27] meeting Chompret criteria[13] in which 2 relatives carrying the p.Arg290His *TP53* alteration were diagnosed as having astrocytomas at ages 29 and 31 years.[27] Another report[28] included a French Canadian woman with breast cancer at age 44 years who carried the p.Arg290His *TP53* alteration; although there were multiple other cancers in this family, they did not meet Chompret criteria.[14,28] One report[29] included a male proband with rhabdomyosarcoma at age 2 years and a brain tumor at 10 years of age who carried the p.Arg290His alteration and 2 other germline *TP53* variants (p.Arg156His and p.Arg267Gln). This patient's mother, who had had metachronous breast cancers at 35 and 43 years of age, was confirmed to carry the p.Arg156His and p.Arg267Gln alterations, and the maternal family history met Chompret[14] criteria. The patient's cancer-free father carried the p.Arg290His alteration.[29] The p.Arg290His *TP53* alteration was also described in a female proband[29] with a brain tumor at age 9 years whose maternal grandfather (mutation status unknown) died of a brain tumor at age 40 years and whose paternal first cousin (mutation status unknown) died of rhabdomyosarcoma at age 4 years.

Participant 4 was a white man with sigmoid CRC at age 25 years, who carried a c.847C>T (p.Arg283Cys) *TP53* alteration. Prior reports of germline p.Arg283Cys *TP53* alterations include a proband with metachronous breast cancers and a subsequent leiomyosarcoma who

carried both the p.Arg283Cys *TP53* alteration and a pathogenic p.Arg2394X alteration in *BRCA2*.[30] This proband's family history included a sister with ovarian cancer at age 39 years, a mother with breast and ovarian cancers at ages 60 and 65 years, respectively, and a daughter with glioblastoma at age 41 years, none of whom had had testing for the *TP53* or *BRCA2* mutations.[30] Another report[31] described a female proband with diffuse-type gastric carcinoma at age 52 years who tested negative for a germline *CDH1* mutation but carried a germline *TP53* p.Arg283Cys alteration. Her family history included a mother and maternal uncle with gastric cancer (ages unknown), a sister with leukemia (age 17 years), and another sister with liver carcinoma (age 34 years), none of whom had been tested for the *TP53* p.Arg283Cys alteration.[31] Other reports of germline p.Arg283Cys *TP53* alteration carriers include a woman[32] with *HER2/neu*-negative breast cancer (age unknown), for whom family history data were unknown, and a 25-year-old proband[33] with a desmoplastic small round cell tumor and no family history of cancer.

Participant 5 was a woman (race unknown) with descending colon adenocarcinoma at age 19 years, who carried a c.850A>T (p.Thr284Ser) *TP53* alteration. To our knowledge, germline p.Thr284Ser *TP53* alterations have not been previously described in the literature, although a somatic p.Thr284Ser *TP53* alteration in a patient with B-cell chronic lymphocytic leukemia has been reported.[34]

Participant 6 was a white woman with CRC at age 35 years, who carried a c.704A>G (p.Asn235Ser) *TP53* alteration. Prior reports of germline p.Asn235Ser *TP53* alterations include that of a proband with an embryonal rhabdomyosarcoma of the vagina at age 19 years, with no family history of cancer.[35] Another report[36] included a female proband with breast cancer at age 26 years, found to carry the germline p.Asn235Ser *TP53* alteration, whose mother had ovarian cancer and whose sister had breast cancer (age and mutation status were unknown for both). The germline p.Asn235Ser *TP53* alteration was found in a Finnish woman[37] with bilateral breast cancer at age 57 years and her nephew with an ependymoma at age 19 years; other members of this family with unknown germline *TP53* status included 3 women with breast cancer at unknown ages and 3 cases of gastric cancer at unknown ages.[37] One large family[38] who met classic LFS criteria[8] was found to carry the p.Asn235Ser alteration as well as an intronic splice site (IVS5-1G>A) *TP53* mutation. The pathogenicity of the p.Asn235Ser alteration was questioned by the report's authors because it did not segregate with cancer phenotype, whereas the IVS5-1G>A mutation did.[38]

Discussion

Overall, 1.3% of this large cohort of patients with early-onset CRC was found to carry germline *TP53* alterations. None of these probands had clinical histories meeting the criteria for *TP53* testing, and 3 of these alterations were confirmed to be carried by the participants' cancer-free parents. Four of these alterations have been previously reported in the literature as germline *TP53* alterations, and at least some of the probands described in such reports had clinical histories consistent with LFS.

Prior studies examining the rates of cancer susceptibility gene mutations in early-onset CRC have typically focused on testing for Lynch syndrome, FAP, and MAP, which are presumed

to be more common causes of early-onset CRC than LFS. In another study[3] of population-based CCFR patients, 5.6% of a random sample of participants with CRC younger than 50 years carried germline mutations in *MLH1*, *MSH2*, or *MSH6*. A retrospective analysis of a cohort of Spanish patients with CRC at 50 years oldor younger found that, after excluding those with polyposis phenotypes, 7.8% carried germline *MLH1*, *MSH2*, or *MSH6* mutations and an additional 2.8% carried biallelic *MUTYH* mutations.[4] Similarly, a retrospective analysis[39] of an American cohort of patients with CRC diagnosed before age 36 years found that, after excluding those with more than 10 colorectal adenomas, 29% carried *MLH1*, *MSH2*, or *MSH6* mutations, although virtually all of these carriers had a personal and/or family history of Lynch-associated cancer. Notably, they also found that 1 of the 96 participants in their cohort carried a germline *TP53* mutation, although systematic *TP53* testing was not otherwise performed.[39] Our work adds to this literature in that it is the first study, to our knowledge, to systematically test patients with early-onset CRC for germline *TP53* mutations. Although at first glance, the fraction of participants (1.3%) found to carry germline *TP53* alterations in our study is small, it is comparable with the proportion of inherited CRC thought to be attributable to germline *APC* mutations.[5]

Another key finding of our study is that none of the *TP53* alteration carriers had personal and/or family histories that met clinical criteria for LFS. Prior studies[40,41] have found a 4% to 5% prevalence of germline *TP53* mutations in population-based cohorts of women with early-onset breast cancer and, similar to our data, found that most mutation carriers do not meet clinical criteria for LFS. Such findings raise the fundamental question as to whether carriage of a germline *TP53* mutation should be pathognomonic for diagnosing LFS. If a substantial fraction of *TP53* mutation carriers indeed fail to meet clinical criteria for LFS, this calls into doubt the assumption that all germline *TP53* alterations confer the 73% to 100% life-time risks of cancer associated with classic LFS[10] and raises important issues regarding the impact of ascertainment on counseling of families found to carry germline alterations.

With the advent of multigene panels, exome sequencing, and other comprehensive strategies for hereditary cancer risk assessment, a growing number of patients will likely undergo germline analysis of *TP53* and other cancer susceptibility genes, even in the absence of classic phenotypic features.[15,42] A predominant concern about such approaches is that they will reveal a large number of patients with germline variant of uncertain significance, most of which will be missense mutations; this can be anxiety-provoking and potentially misinterpreted by both patients and clinicians.[15,42]

All 6 of the *TP53* alterations identified in this study were missense mutations, thus raising questions regarding their pathogenicity. Three features of *TP53* mutations in LFS make missense variant of uncertain significance (VUS) assessment particularly challenging. First, de novo alterations are thought to account for as many as 20% of pathogenic *TP53* mutations, which thus prevents reassurance when a *TP53* alteration carrier lacks a family history of cancer.[43] Second, a disproportionate majority of pathogenic *TP53* mutations are missense mutations, rather than nonsense mutations, splice site mutations, or insertion/deletions.[10,44,45] Third, there are compelling data to suggest that missense *TP53* mutations may actually be more oncogenic than other types of loss-of-function *TP53* mutations,

thereby highlighting the importance of appropriate pathogenicity assessment for any *TP53* missense alterations.[10,44] Notably, 5 of the *TP53* alterations identified in our study were in the gene's DNA-binding domain (codons 94–292), which is where most LFS-causing mutations are found.[10]

Our study's primary strength is its use of a large, multicenter cohort of patients with CRC recruited through both clinic-based and population-based means, which serves to limit potential ascertainment bias. The availability of detailed family history data collected in a uniform fashion by cancer genetics researchers, allowed assessment of whether the families met clinical criteria for various hereditary CRC syndromes. The use of centralized *TP53* mutation analysis, so as to minimize procedural errors, is another strength.

We acknowledge that our study has limitations. We are unable to verify the completeness and accuracy of reported family histories since the initiation of the study, and it is unknown if family members may have subsequently developed LFS-associated tumors, which is particularly important owing to the young age of the probands studied. Furthermore, because participants with known hereditary CRC syndromes were excluded, we are unable to claim that our study defines the true prevalence of *TP53* alterations in patients with early-onset CRC. Because many participants in our study had not previously undergone Lynch syndrome testing, it is possible that this cohort included patients with undiagnosed Lynch syndrome. If indeed a fraction of our cohort has unrecognized Lynch syndrome, then the true prevalence of *TP53* mutations in patients with early-onset CRC without Lynch syndrome would actually be higher than the 1.3% rate observed in this study. The true prevalence of most hereditary CRC syndromes remains undefined, because most prior large studies have involved some sort of clinical preselection, rather than population-based testing. Thus, to precisely define the prevalence of patients with CRC with cancer susceptibility gene mutations, future studies using multiplex panel testing, whole genome sequencing, or some other form of comprehensive germline analysis will be needed.

Our *in silico* assessments and literature searches are imperfect tools to interpret the clinical and biologic significance of identified *TP53* alterations, and we are thus unable to precisely classify their pathogenicity or determine whether they are truly the etiologic basis of the observed early-onset CRC. Although this is admittedly another limitation of our study, it also highlights a prominent real-world challenge that clinicians will face with more widespread *TP53* testing in patients lacking classic LFS phenotypes. The typical management for *TP53* mutation carriers involves aggressive and early radiographic, laboratory, endoscopic, and sometimes even surgical risk-reduction strategies, based on the notion that *TP53* mutations confer a LFS phenotype with a near-100% lifetime risk of malignant disease.[10,46,47] Our study was unable to define the penetrance of our participants' *TP53* alterations, although these findings raise the hypothesis that some pathogenic *TP53* mutations do not confer a classic LFS phenotype.

Conclusions

To our knowledge, this report represents the largest study to date examining germline *TP53* alterations in individuals with early-onset CRC. Given that none of the *TP53* probands in

our study met clinical criteria for LFS, our data raise the question as to whether LFS should be defined by the presence of pathogenic germline *TP53* mutations. This reflects a growing quandary in the field of cancer genetics, which, in recent years, has shifted toward using genotypic data (eg, carriage of a germline MMR mutation in Lynch syndrome) to define and diagnose specific hereditary cancer syndromes rather than the historical practice of using phenotypic information (eg, fulfillment of Amsterdam criteria in Lynch syndrome). Newer studies consistently show, however, that a subset of patients with germline mutations in MMR genes, *APC*, *MUTYH*, and now *TP53*, have particularly attenuated clinical histories, calling into question whether management recommendations should take both genotype and phenotype into account.[48,49]

For patients found to carry *TP53* mutations in the setting of early-onset CRC but no other clinical features of LFS, our data suggest that clinicians may be able to reassure such probands that the lifetime risk of classic LFS cancers may not be as high as the 73% to 100% risk typically quoted, although confirmatory studies are certainly needed. These findings highlight the inevitable challenges raised by comprehensive approaches to hereditary cancer risk assessment, namely, the interpretation of missense alterations and VUS in cancer susceptibility genes, as well as the difficulties in estimating future cancer risks in families with atypical phenotypes. With modern techniques for comprehensively genotyping cancer patients, interpreting such germline results will undoubtedly be a prominent challenge in the counseling and management of at-risk individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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At a Glance

- Individuals with Li-Fraumeni syndrome (LFS) caused by germline *TP53* mutations are estimated to have a 73% to 100% lifetime risk of cancer, including colorectal cancer (CRC).
- The purpose of this study was to determine the frequency of germline TP53 alterations in individuals with early-onset CRC.
- Of 457 participants diagnosed as having CRC at age 40 years or younger, 1.3% carried germline *TP53* alterations.
- None of the *TP53* probands in this study had a personal or family cancer history that fulfilled clinical LFS criteria.
- Cancer risk in *TP53* mutation carriers may be different in patients presenting with early-onset CRC compared with those who present with classic LFS family histories.

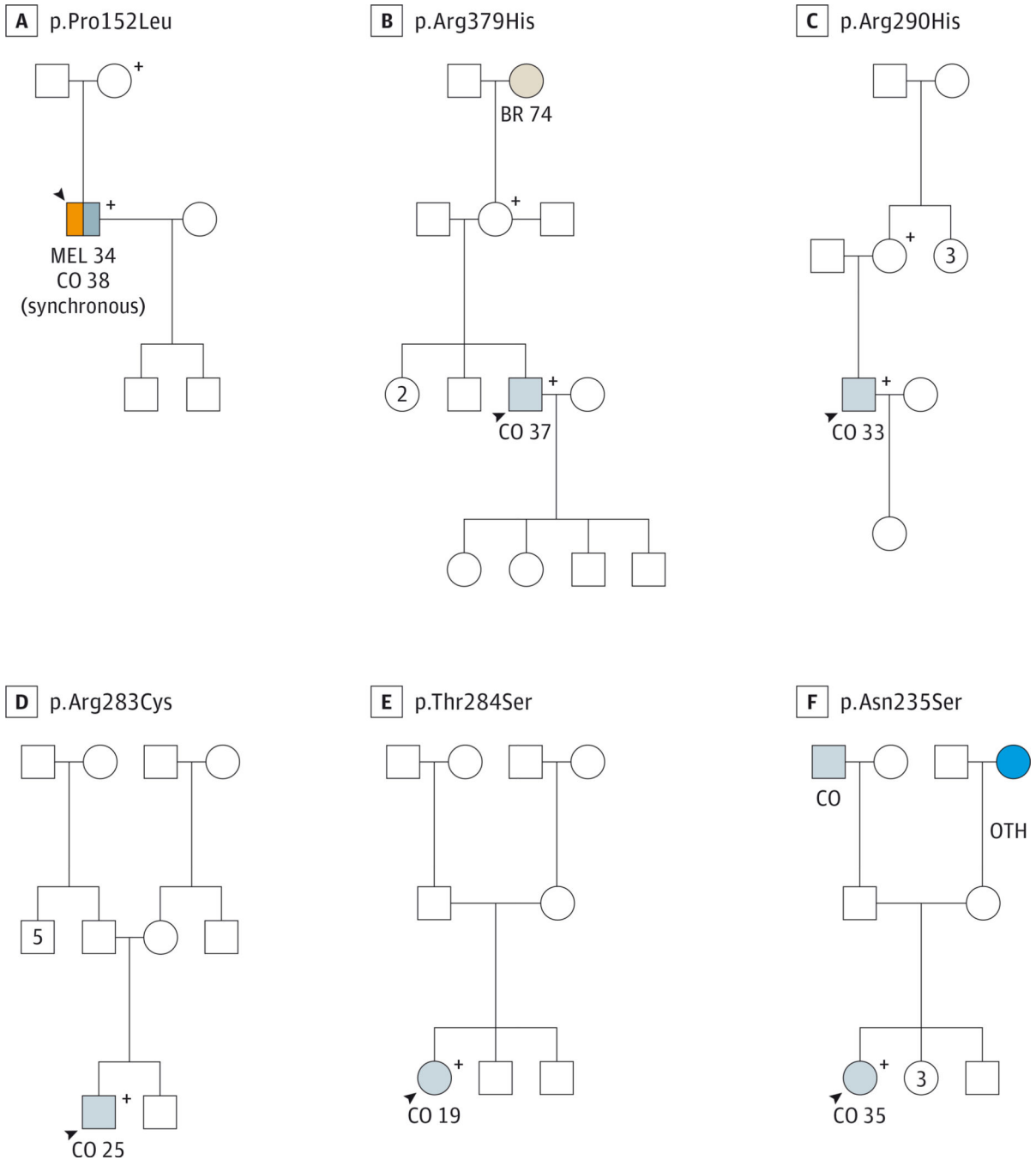

Figure. Pedigrees of 6 Participants With Early-Onset Colorectal Cancer Found to Carry Germline *TP53* Alterations

A–F, Panels represent patients 1 to 6, respectively; see Results section. BR indicates breast cancer; CO, colorectal cancer; MEL, melanoma; OTH, other cancer. Squares represent male family members, and circles represent female family members. Numbers represent age in years at diagnosis. The numbers and letters at the top of each panel indicate the specific germline *TP53* mutation carried by the family described in each panel. Plus signs indicate that the individual was confirmed to carry the germline *TP53* alteration. Shading indicates

that the individual was affected with cancer. The arrowheads indicate the specific study participant for that family.

Table 1

Characteristics of 457 Participants Diagnosed as Having Colorectal Cancer at Age 40 Years or Younger

Characteristic	No. (%)
Sex	
Male	204 (45)
Female	253 (55)
Race	
White	382 (84)
Black/African American	11 (2)
Hispanic	7 (2)
Asian	16 (4)
Native American	1 (<1)
Middle Eastern	4 (1)
Other	3 (1)
Missing/unknown	33 (7)
Cancer history	
1 CRC only	389 (85)
1 CRC + other cancer(s)	34 (7)
Synchronous/metachronous CRCs only	26 (6)
Synchronous/metachronous CRCs + other cancer(s)	8 (2)
Site of colorectal cancer	
Right colon	121 (26)
Left colon	105 (23)
Rectum	149 (33)
Appendix	7 (2)
Missing/unknown	75 (16)
MSI and MMR IHC status[a]	
MSI-H and/or abnormal MMR IHC	47 (10)
MSS/MSI-L and/or normal MMR IHC	279 (61)
Missing tumor testing data	131 (29)
Prior genetic testing results (Lynch genes and/or *MUTYH*)[b]	
Prior genetic testing of ≥1 Lynch genes	162 (35)
Negative result for *MLH1* testing	142 (31)
MLH1 VUS detected	12 (3)
Negative result for *MSH2* testing	129 (28)
MSH2 VUS detected	2 (<1)
Negative result for *MSH6* testing	40 (9)
MSH6 VUS detected	2 (<1)
Negative result for *PMS2* testing	22 (5)

Characteristic	No. (%)
PMS2 VUS detected	1 (<1)
Negative result for *MUTYH* testing	387 (85)
Monoallelic *MUTYH* mutation detected	10 (2)
MUTYH VUS detected	0
Recruitment method	
Population-based	314 (69)
Clinic-based	143 (31)

Abbreviations: CRC, colorectal cancer; MSI, microsatellite instability; MMR IHC, mismatch repair immunohistochemistry testing; MSI-H, high-level microsatellite instability; MSI-L, low-level microsatellite instability; MSS, microsatellite stable; VUS, variant of uncertain significance.

[a] No participants had discordant MSI and MMR IHC results.

[b] Rows are not mutually exclusive; numerous participants had prior genetic testing for more than 1 gene.

Table 2

Characteristics of *TP53* Alterations Identified Among 457 Participants With Colorectal Cancer (CRC) at Age 40 Years or Younger[a]

Patient No./ Sex/Age[b]	Family History		Nucleotide Change	Genome Loc	Amino Acid Change	Minor Allele Freq	Species Conservation	Exon	*In silico* Pathogenicity Assessment Results[c]	
	Chompret Crit	Classic LFS Crit							PolyPhen-2	SIFT
1/M/38	No	No	c.455C>T	g.7578475	p.Pro152Leu	Undefined	Highly conserved	5	Probably damaging	Not tolerated
2/M/37	No	No	c.1136G>A	g.7572973	p.Arg379His	Undefined	Moderately conserved	11	Benign	Tolerated
3/M/33	No	No	c.869G>A rs55819519	g.7577069	p.Arg290His	0.0003EA 0.0002AA	Moderately conserved	8	Benign	Tolerated
4/M/25	No	No	c.847C>T rs149633775	g.7577091	p.Arg283Cys	0.0005EA 0.0002AA	Moderately conserved	8	Benign	Not tolerated
5/F/19	No	No	c.850A>T	g.7577088	p.Thr284Ser	Undefined	Moderately conserved	8	Possibly damaging	Tolerated
6/F/35	No	No	c.704A>G rs114340710	g.7577577	p.Asn235Ser	0.0001EA 0.0002AA	Moderately conserved	7	Benign	Tolerated

Abbreviations: AA, African American; crit, criteria; EA, European American; freq, frequency; LFS, Li-Fraumeni syndrome; loc, location.

[a] None of the 6 *TP53* probands carried a known germline variant of uncertain significance in *MLH1*, *MSH2*, *MSH6*, or *PMS2*; none carried a monoallelic *MUTYH* mutation.

[b] Age in years at CRC diagnosis.

[c] Interactive Biosoftware.