Supplementary table 1. Characteristics of patients with Lynch syndrome.

Characteristic	Germline MMR gene mutation (n=312)			
	MLH1	***MSH2***	***MSH6***	***PMS2***
Probands –no.	114	129	40	29
Male gender –no. (%)	38/82 (46.3)	46/93 (49.5)	24/32 (75.0)	17/28 (60.7)
Age (years.)[a]	47.2 ± 11.7	46.4 ± 12.2	53.8 ± 16.6	50.1 ± 12.6
Proximal CRC –no. (%)[b,c]	55/78 (70.5)	50/86 (58.1)	10/23 (43.5)	14/20 (70.0)
Mucinous CRC –no. (%)[b]	16/78 (20.5)	24/81 (29.6)	7/24 (29.2)	5/18 (27.8)
Synchronous or metachronous CRC –no. (%)[b]	21/86 (24.4)	23/95 (24.2)	5/35 (15.6)	1/28 (3.6)
Metachronous Lynch syndrome-related tumor (CRC excluded) –no. (%)[b,d]	6/114 (5.3)	12/129 (9.3)	- (-)	3/29 (10.3)
At least one first-degree relative with CRC –no. (%)[b]	57/113 (50.4)	64/126 (50.8)	5/40 (12.5)	5/29 (17.2)
Fulfillment of at least one criterion of revised Bethesda guidelines –no. (%)[b]	83/86 (96.5)	89/97 (91.8)	19/32 (59.4)	23/28 (82.1)
Fulfillment of Amsterdam I criteria –no. (%)[b]	17/97 (17.5)	21/125 (16.8)	2/40 (5.0)	1/29 (3.4)
Fulfillment of Amsterdam II criteria –no. (%)[b]	40/114 (35.1)	42/129 (32.6)	2/40 (5.0)	1/29 (3.4)
Loss of MMR protein expression –no. (%)[b]	75/80 (93.8)	117/121 (96.7)	29/32 (90.6)	28/29 (96.5)
Tumor MSI-high –no. (%)[b]	73/76 (96.0)	88/92 (95.7)	24/26 (92.3)	20/21 (95.2)

MMR, mismatch repair; CRC, colorectal cancer; MSI, microsatellite instability.
[a]Expressed as mean ± standard deviation.
[b]Probands fulfilling the corresponding condition (reflected in the numerator) with respect to those in whom this variable could be evaluated (reflected in the denominator).
[c]With respect to the splenic flexure.
[d]Lynch syndrome-related tumors: colorectal, endometrial, ovarian, gastric, hepatobiliary, small-bowel, urinary tract, pancreatic, and brain cancer.

Supplementary table 2. Distribution of germline mismatch repair gene mutations according to the age at colorectal cancer diagnosis[a].

Age at diagnosis	Evaluable probands –no.	Germline MMR gene mutation –no. (%)	Distribution of patients with mutations according to age at diagnosis	Distribution of patients with mutations according to age at diagnosis (cumulative)
<15 years	0	0 (0.0)	-	-
15 to 20 years	1	0 (0.0)	-	-
21 to 25 years	4	1 (25.0)	1.2%	1.2%
26 to 30 years	16	3 (18.7)	3.7%	4.9%
31 to 35 years	29	7 (24.1)	8.5%	13.4%
36 to 40 years	67	7 (10.4)	8.5%	21.9%
41 to 45 years	111	7 (6.3)	8.5%	30.5%
46 to 50 years	212	12 (5.7)	14.6%	45.1%
51 to 55 years	299	4 (1.3)	4.9%	50.0%
56 to 60 years	351	15 (4.3)	18.3%	68.3%
61 to 65 years	449	8 (1.8)	9.8%	78.0%
66 to 70 years	586	6 (1.0)	7.3%	85.4%
71 to 75 years	611	5 (0.8)	6.1%	91.5%
76 to 80 years	518	3 (0.6)	3.7%	95.1%
81 to 85 years	275	4 (1.4)	4.9%	100%
86 to 90 years	118	0 (0.0)	-	-
91 to 95 years	18	0 (0.0)	-	-
96 to 100 years	4	0 (0.0)	-	-
>100 years	2	0 (0.0)	-	-
Total	**3,671**	**82 (2.2)**	**100%**	**100%**

MMR, mismatch repair.
[a]This analysis was limited to population-based cohorts (n=3,671 probands).

Supplementary table 3. Performance characteristics of selected strategies for the identification of patients with Lynch syndrome in the overall series[a].

Selected strategy		Evaluable probands[b] –no.	Probands requiring tumor MMR testing[c] –no. (%)	Probands requiring germline MMR gene analysis[d] –no. (%)	Germline MMR gene mutation							
					Se –no.; % (95% CI)	Sp –no.; % (95% CI)	PPV –no.; % (95% CI)	NPV –no.; % (95% CI)	Diagnostic yield[e] –no.; % (95% CI)	p-value[f]	Incremental diagnostic yield[g] –%	False positive yield[h] –no.; % (95% CI)
Tumor MMR testing in CRC patients fulfilling the following condition:	At least one criterion of revised Bethesda guidelines	8,644	3,886 (45.0)	547 (6.3)	202/233; 86.7% (82.1-91.2%)	8,066/8,411; 95.9% (95.4-96.3%)	202/547; 36.9% (32.7-41.0%	8,066/8,097; 99.6% (99.4-99.7%)	202/8,644; 2.3% (2.0-2.6%)	<.001	-	345/8,644; 4.0% (3.5-4.4%)
	Multivariate model[j]	8,612	4,579 (53.2)	502 (5.8)	206/233; 88.4% (84.0-92.7)	8,083/8,379; 96.5% (96.0-96.8%)	206/502; 41.0% (36.6-45.4%)	8,083/8,110; 99.7% (99.5-99.8%)	206/8,612; 2.4% (2.0-2.7%)	<.001	0.06	296/8,612; 3.4% (3.0-3.8%)
	Jerusalem recommendations[i]	8,591	6,658 (77.5)	698 (8.1)	211/229; 92.1% (88.4-95.8%)	7,875/8,362; 94.2% (93.6-94.6%)	211/698; 30.2% (26.4-33.7%)	7,875/7,893; 99.8% (99.6-99.9%)	211/8,591; 2.5% (2.1-2.8%)	.04	0.06	487/8,591; 5.6% (5.1-6.1%)
	Jerusalem recommendations[i] or at least one criterion of revised Bethesda guidelines	8,638	7,053 (81.7)	777 (9.0)	223/233; 95.7% (92.8-98.5%)	7,851/8,405; 93.4% (92.8-93.9%)	223/777; 28.7% (25.4-31.9%)	7,851/7,861; 99.9% (99.7-99.9%)	223/8,638; 2.6% (2.2-2.9%)	.09	0.1	554/8,638; 6.4% (5.9-6.9%)
Tumor MMR testing in any given CRC patient (universal strategy)		9,701	9,701 (100)	1,068 (11.0)	289/301; 96.0% (93.6-98.3%)	8,621/9,400; 91.7% (91.1-92.2%)	289/1,068; 27.1% (24.3-29.7%)	8,621/8,633; 99.9% (99.7-99.9%)	289/9,701; 2.9% (2.6-3.3%)	Ref.	0.4	779/9,701; 8.0% (7.4-8.5%)

MMR, mismatch repair; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; 95% CI, 95% confidence interval; CRC, colorectal cancer.

[a]This analysis was done including both population- and non-population-based cohorts.

[b]Probands in whom the corresponding strategy could be assessed.

[c]Probands requiring tumor MMR testing in each strategy, with respect to those in whom it could be assessed.

[d]Probands requiring germline MMR gene analysis because of the demonstration of tumor MMR deficiency in each strategy, with respect to those in whom it could be assessed.

[e]Diagnostic yield refers to probands requiring germline MMR gene analysis in whom a mutation was found.

[f]Matthews correlation coefficient comparison of diagnostic yield with respect to the universal strategy.

[g]Compared with the next least intensive strategy.

[h]False positive yield refers to probands requiring germline MMR gene analysis in whom no mutation was found.

[i]Age at CRC diagnosis ≤70 years-old.

[j]Defined as fulfillment of at least one of the following characteristics: CRC diagnosed at age of 60 or younger, at least one first-degree relative with CRC diagnosed at age of 50 or younger, or personal history of metachronous Lynch syndrome-related tumors diagnosed at age of 50 or younger.

Supplementary table 4. Performance characteristics of selected strategies for the identification of patients with Lynch syndrome, according to the mismatch repair gene mutated in overall series[a]

MMR gene	Selected strategy		Evaluable probands[b] –no.	Probands requiring tumor MMR testing[c] –no. (%)	Probands requiring germline MMR gene analysis[d] –no. (%)	Germline MMR gene mutation						
						Se –no.; % (95% CI)	Sp –no. ; % (95% CI)	PPV –no. ; % (95% CI)	NPV –no. ; % (95% CI)	PLR (95% CI)	NLR (95% CI)	p-value[e]
MLH1	Tumor MMR testing in CRC patients fulfilling the following condition:	At least one criterion of revised Bethesda guidelines	8,357	3,676 (43.9)	338 (4.0)	77/81; 95.1% (89.7-100%)	8,015/8,276; 96.8% (96.4-97.2%)	77/338; 22.8% (18.1-27.4%)	8,015/8,019; 100% (99.9-100%)	29.6 (26.4-34.3)	0.05 (0.02-0.1)	<.001
		Jerusalem recommendations[f]	8,307	6,397 (77.0)	439 (5.3)	76/80 ; 95.0% (89.6-100%)	7,864/8,227; 95.6% (95.1-96.0%)	76/439; 17.3% (13.6-20.9%)	7,864/7,868; 99.9% (99.8-100%)	21.5 (19.2-24.0)	0.05 (0.02-0.1)	<.001
		Jerusalem recommendations[f] or at least one criterion of revised Bethesda guidelines	8,351	6,781 (81.2)	507 (6.1)	79/81; 97.5% (93.5-100%	7,842/8,270; 94.8% (94.3-95.3%)	79/507; 15.6% (12.3-18.8%)	7,842/7,844; 100% (99.9-100%)	18.7 (17.0-20.8)	0.02 (0.01-0.1)	<.001
		Multivariate model[g]	8,325	4,354 (52.3)	279 (3.4)	73/81; 90.1% (83.0-97.2%)	8,038/8,244; 97.5% (97.1-97.8%)	73/279; 26.2% (20.8-31.5%)	8,038/8,046; 99.9% (99.8-99.9%)	36.0 (30.9-42.0)	0.1 (0.05-0.2)	<.001
	Tumor MMR testing in any given CRC patient (universal strategy)		9,347	9,347 (100)	722 (7.7)	103/108; 95.4% (90.9-99.8%)	8,620/9,239; 93.3% (92.7-93.8%)	103/722; 14.3% (11.6-16.8%)	8.620/8,625; 99.9% (99.8-100%)	14.2 (130-15.5)	0.05 (0.02-0.1)	Ref.
MSH2	Tumor MMR testing in CRC patients fulfilling the following condition:	At least one criterion of revised Bethesda guidelines	7,938	3,499 (44.0)	162 (2.0)	88/96; 91.7% (85.6-97.7%)	7,768/7,842; 99.1% (98.8-99.2%)	88/162; 54.3% (46.3-62.3%)	7,768/7,776; 99.9% (99.8-99.9%)	101.8 (76.8-122.8)	0.08 (0.04-0.1)	<.001
		Jerusalem recommendations[f]	7,892	6,138 (77.7)	180 (2.3)	88/93; 94.6% (89.5-99.7%)	7,707/7,799; 98.8% (98.5-99.0%)	88/180; 48.9% (41.3-56.4%)	7,707/7,712; 99.9% (99.8-100%)	78.8 (65.1-98.8)	0.05 (0.02-0.1)	<.001
		Jerusalem recommendations[f] or at least one criterion of revised Bethesda guidelines	7,933	6,470 (81.5)	196 (2.5)	95/96; 99.0% (96.4-100%)	7,736/7,837; 98.7% (98.4-98.9%)	95/196; 48.5% (41.2-55.7%)	7,736/7,737; 100% (99.9-100%)	76.1 (63.1-93.3)	0.01 (0.0-0.07)	<.001
		Multivariate model[g]	7,910	4,233 (53.5)	158 (2.0)	91/96; 94.8% (89.8-99.7%)	7,747/7,814; 99.1% (98.9-99.3%)	91/158; 57.6% (49.5-65.6%)	7,747/7,752; 99.9% (99.8-100%)	105.3 (86.7-140.9)	0.05 (0.02-0.1)	<.001
	Tumor MMR testing in any given CRC patient (universal strategy)		8,896	8,896 (100)	273 (3.1)	125/128; 97.7% (94.6-100%)	8,620/8,768; 98.3% (98.0-98.6%)	125/273; 45.8% (39.6-51.8%)	8,620/8,623; 100% (99.9-100%)	54.1 (49.2-68.0)	0.02 (0.01-0.07)	Ref.
MSH6	Tumor MMR testing in CRC patients fulfilling the following condition:	At least one criterion of revised Bethesda guidelines	7,806	3,382 (43.3)	47 (0.6)	14/28; 50.0% (29.7-70.3%)	7,745/7,778; 99.6% (99.4-99.7%)	14/47; 29.8% (15.6-43.9%)	7,745/7,759; 99.8% (99.7-99.9%)	125.0 (71.2-194.9)	0.5 (0.3-0.7)	<.001
		Jerusalem recommendations[f]	7,765	6,016 (77.4)	62 (0.8)	22/28; 78.6% (61.6-95.5%)	7,697/7,737; 99.5% (99.3-99.6%)	22/62; 35.5% (22.7-48.2%)	7,697/7,703; 99.9% (99.8-99.9%)	157.2 (105.5-218.8)	0.2 (0.1-0.4)	.537
		Jerusalem recommendations[f] or at least one criterion of revised Bethesda guidelines	7,803	6,342 (81.2)	73 (0.9)	23/28; 82.1% (66.1-98.1%)	7,725/7,775; 99.3% (99.1-99.5%)	23/73; 31.5% (20.1-42.8%)	7,725/7,730; 99.9% (99.8-100%)	117.0 (92.2-176.9)	0.1 (0.08-0.4)	.257

		Multivariate model[g]	7,780	4,118 (52.9)	47 (0.6)	17/28; 60.7% (40.8-80.6%)	7,722/7,752; 99.6% (99.4-99.7%)	17/47; 36.2% (21.3-50.9)	7,722/7,733; 99.9% (99.7-99.9)	151.7 (98.5-249.8)	0.4 (0.2-0.6)	<.001
	Tumor MMR testing in any given CRC patient (universal strategy)		8,729	8,729 (100)	111 (1.3)	33/36; 91.7% (81.2-100%)	8,615/8,693; 99.1% (98.9-99.3%)	33/111; 29.7% (20.7-38.8%)	8,615/8,618; 100% (99.9-100%)	101.8 (80.2-130.1)	0.08 (0.03-0.2)	Ref.
PMS2	Tumor MMR testing in CRC patients fulfilling the following condition:	At least one criterion of revised Bethesda guidelines	7,756	3,369 (43.4)	37 (0.5)	23/28; 82.1% (66.1-98.1%)	7,714/7,728; 99.8% (99.7-99.9%)	23/37; 62.2% (45.1-79.1%)	7,714/7,719; 99.9% (99.8-100%)	410.5 (261.3-786.7)	0.1 (0.08-0.4)	.096
		Jerusalem recommendations[f]	7,719	5,996 (77.6)	44 (0.6)	25/28; 89.3% (76.0-100%)	7,672/7,691; 99.8% (99.6-99.9%)	25/44; 56.8% (41.0-72.6%)	7,672/7,675; 100% (99.9-100%)	446.5 (226.5-576.5)	0.1 (0.04-0.3)	.161
		Jerusalem recommendations[f] or at least one criterion of revised Bethesda guidelines	7,756	6,312 (81.3)	46 (0.6)	26/28; 92.9% (81.5-100%)	7,708/7,728; 99.7% (99.6-99.8%)	26/46; 56.5% (41.1-71.9%)	7,708/7,710; 100% (99.9-100%)	309.6 (228.8-562.4)	0.07 (0.02-0.2)	.002
		Multivariate model[g]	7,734	4,109 (53.1)	40 (0.5)	25/28; 89.3% (76.0-100%)	7,691/7,706; 99.8% (99.7-99.9%)	25/40; 62.5% (46.2-78.7%)	7,691/7,694; 100% (99.9-100%)	446.5 (272.2-772.7)	0.1 (0.04-0.3)	<.001
	Tumor MMR testing in any given CRC patient (universal strategy)		8,662	8,662 (100)	55 (0.6)	28/29; 96.6% (88.2-100%)	8,606/8,633; 99.7% (99.5-99.8%)	28/55; 50.9% (36.8-65.0%)	8,606/8,607; 100% (99.9-100%)	322.0 (210.5-452.7)	0.03 (0.01-0.2)	Ref.

MMR, mismatch repair; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; 95% CI, 95% confidence interval; CRC, colorectal cancer.

[a]This analysis was done including both population- and non-population-based cohorts.

[b]Probands in whom the corresponding strategy could be assessed.

[c]Probands requiring tumor MMR testing in each strategy, with respect to those in whom it could be assessed.

[d]Probands requiring germline MMR gene analysis because of the demonstration of tumor MMR deficiency in each strategy, with respect to those in whom it could be assessed.

[e]With respect to universal strategy (comparing Mathews correlation coefficients).

[f]Age at CRC diagnosis ≤70 years-old.

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