Supplementary Online Content

Karam R, Conner B, LaDuca H, et al. Assessment of diagnostic outcomes of RNA genetic testing for hereditary cancer. *JAMA Netw Open*. 2019;2(10):e1913900. doi:10.1001/jamanetworkopen.2019.13900

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. RNA Clinical Management Survey

Q1 Please select the response which describes the type of variant reclassification that occurred:

- O Downgraded from VUS to Benign/Likely Benign
- O Upgraded from VUS to Pathogenic/Likely Pathogenic
- O Downgraded from Pathogenic/Likely Pathogenic to VUS

Q2 Did the reclassification change your **medical management** recommendations for the patient?

- O Yes
- O No (If no, skip to Q5)

Q3 Which of the following best describes the change in your **medical management** recommendations for the patient?

- O Recommended **increased** cancer screening and/or risk-reducing interventions
- O Recommended **decreased** cancer screening and/or recommended **against** risk-reducing interventions

Q4 Please provide details regarding how your recommendations for cancer screening and/or risk-reducing interventions changed for the patient:

_____

_____

_____

_____

_____

Q5 Did the reclassification change your **medical management** recommendations for the patient's family members?

O Yes

O No (If no, skip to Q8)

Q6 Which of the following best describes the change in your **medical management** recommendations for the patient's family members?

O Recommended **increased** cancer screening and/or risk-reducing interventions

O Recommended **decreased** cancer screening and/or recommended against risk-reducing interventions

Q7 Please provide details regarding how your recommendations for cancer screening and/or risk-reducing interventions changed for the patient's family members:

_____

_____

_____

_____

_____

Q8 Did the reclassification change your **genetic testing** recommendations for the patient's family members?

O Yes

O No (If no, skip to Q10)

Q9 Which of the following describes the change in your **genetic testing** recommendations for the patient's family members?

- O Recommended genetic testing for patient's family members for reclassified variant
- O Recommended **against** genetic testing for patient's family members for reclassified variant
- O Other (please describe): _____

Q10 Please use this space to provide any additional comments regarding the impact of reclassification on the patient and his/her family:

_____

_____

_____

_____

_____

Q11 Thank you for completing the survey. Would you like to enter a raffle for the chance to win a gift card?

- O Yes
- O No

eTable. Summary of Variants Analyzed by RGT

DNA Variant	RNA Evidence	Splicing in silico	Assertion Before RGT	Final Assertion	Evidence (ACMG/AMP Codes)	Clinically Actionable Reclassification	Total Patients impacted to date
ATM c.73-3C>G	r.73_79del7	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (Absent from population controls (PM2) *in silico* splicing models predict weakening of the native site (PP3)	y	2
ATM c.8850+5A>C	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict no effect on the native site (BP4)		1
BRCA1 c.80+5G>A	r.-19_80del100; r.-25_80del105	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Co-segregation with disease (PP1_Moderate) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	2
BRCA1 c.594-2A>C; BRCA1 c.641A>G	r.548_670del123 (AS)	Deleterious (TP)[1]	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Observed in healthy individuals (BS2) *in silico* splicing models predict a weakening of the native site (PP3)		32
BRCA1 c.670+1G>T	r.548_670del123 (AS)	Deleterious (TP)	LP	VUS	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict a weakening of the native site (PP3)	y	6
BRCA1 c.3758C>G (p.S1253C)	WT	Deleterious (FP)	VUS	VUS[2]	RNA studies demonstrate no abnormal splicing (BS3) (splice)		n/a

					in silico splicing models predict a weakening of the native site (PP3) (splice)		
BRCA1 c.4357+4T>C	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict no effect on the native site (BP4) Absent from population controls (PM2)		1
BRCA1 c.5072C>A (p.T1691K)	WT	Benign (TN)	VUS	LP[3]	RNA studies demonstrate abnormal splicing (PS3) Located in a mutational hotspot (PM1) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	3
BRCA1 c.5152+5G>T	r.5075_5152del78	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Novel missense change where a different missense change is pathogenic (PM5) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	2
BRCA1 c.5193G>A (p.E1731E)	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict no effect on the native site (BP4)		4
BRCA1 c.5332G>A (p.D1778N)	r.5278_5332del55	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Co-segregation with disease (PP1_Moderate) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	11
BRCA2 c.426-12_426-8delGTTTT	r.426_475del50	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	3
BRCA2 c.681+2dupT	r.632_681del50; r.517_681del165	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	4

BRCA2 c.681+5G>C	r.632_681del50	Deleterious (TP)	VUS	VUS	RNA studies demonstrate abnormal splicing (PS3)_moderate Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		n/a
BRCA2 c.8754+4A>T	r.8754+1_8754+47ins47	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Novel missense change where a different missense change is pathogenic (PM5) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	7
BRCA2 c.9501+3A>T	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Observed in trans with a pathogenic mutation in an individual without biallelic disease (BP2)[4] *in silico* splicing models predict a weakening of the native site (PP3)		84
BRIP1 c.2492+2dupT	r.2493_2575del83	Deleterious (TP)	LP	LP	RNA studies demonstrate abnormal splicing (PS3) Observed in trans with a pathogenic variant in an individual with biallelic disease (PM3) *in silico* splicing models predict a weakening of the native site (PP3)		7
PALB2 c.211+4A>G	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		1
PALB2 c.2379C>T (p.G793G)	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict a weakening of the native site (PP3)		56
PALB2 c.2559C>T (p.G853G)	r.2558_2586del29	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) *in silico* splicing models predict a weakening of the native site (PP3)	y	6
PALB2 c.3113+5G>C	r.3083_3113del31	Deleterious (TP)	LP	LP	RNA studies demonstrate abnormal splicing (PS3) Observed in trans with a pathogenic variant in an individual with biallelic disease (PM3)		n/a

					Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		
PALB2 c.3350+4A>C	r.3202_3350del149	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Novel missense change where a different missense change is pathogenic (PM5) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	3
PALB2 c.3350+5G>A	r.3202_3350del149	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) *in silico* splicing models predict a weakening of the native site (PP3)	y	9
RAD50 c.214-5C>T	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict no effect on the native site (BP4)		4
RAD50 c.2524+4A>T	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict a weakening of the native site (PP3)		5
RAD50 c.2829+5G>C	r.2734_2829del96	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	1
RAD51C c.571+4A>G	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict a weakening of the native site (PP3)		18
RAD51D c.144+3G>T	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict a weakening of the native site (PP3)		6
RAD51D c.738G>A (p.V246V)	c.668_738del71	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	1

RAD51D c.145-4_145-3delGCinsTT	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		3
CDH1 c.164T>G (p.V55G)	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Observed in healthy individuals (BS2) Observed in trans with a pathogenic mutation in an individual without biallelic disease (BP2) *in silico* splicing models predict no effect on the native site (BP4)		38
CDH1 c.387G>T (p.Q129H)	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3)		1
CDH1 c.387+1G>A	r.229_387del159	Deleterious (TP)	LP	VUS	Null variant (PVS1_Moderate) RNA studies demonstrate abnormal splicing (PS3)_supporting Observed in healthy individuals (BS2)_supporting	y	4
CDH1 c.387+5G>A	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Observed in healthy individuals (BS2) *in silico* splicing models predict a weakening of the native site (PP3)		14
CDH1 c.388-4T>C	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Observed in healthy individuals (BS2) *in silico* splicing models predict no effect on the native site (BP4)		5
CDH1 c.558C>T (p.G186G)	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Synonymous change with no predicted splice defect (BP7) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		1
CDH1 c.635G>T (p.G212V)	WT	Benign (TN)	VUS	VUS	RNA studies demonstrate no abnormal splicing (BS3)		n/a

					in silico splicing models predict no effect on the native site (BP4) Proband counting (PS4_Supporting) Absent from population controls (PM2)		
CDH1 c.687+5G>C	r.903_687del42	Deleterious (TP)	VUS	VUS	RNA studies demonstrate abnormal splicing (PS3)_supporting Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		n/a
CDH1 c.921A>G (p.Q307Q)	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Observed in healthy individuals (BS2) *in silico* splicing models predict no effect on the native site (BP4) Synonymous change with no predicted splice defect (BP7) Absent from population controls (PM2)		4
CDH1 c.1008G>T (p.E336D)	r.1008+1_1008+7ins7	Deleterious (TP)	LP	P	Null variant (PVS1_Moderate) RNA studies demonstrate abnormal splicing (PS3) Proband counting (PS4_Moderate) Absent from population controls (PM2) Co-segregation with disease (PP1_Moderate) *in silico* splicing models predict a weakening of the native site (PP3)		2
CDH1 c.1057G>A (p.E353K)	r.1055_1137del83	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Proband counting (PS4_Moderate) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	2
CDH1 c.1137+1delG	r.1055_1137del83	Deleterious (TP)	LP	P	Null variant (Null variant (PVS1_Strong) RNA studies demonstrate abnormal splicing (PS3) Proband counting (PS4_Supporting) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site Known pathogenic variant affecting the same splice site (PP3_Moderate: using c.1137G>A)		7

CDH1 c.1138-5T>G	WT	Deleterious (FN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		1
CDH1 c.1296C>G (p.N432K)	WT	Benign (TN)	VUS	VUS	RNA studies demonstrate no abnormal splicing (BS3) Observed in healthy individuals (BS2_Supporting) *in silico* splicing models predict no effect on the native site (BP4)		n/a
CDH1 c.1703C>G (p.T568R)	r.1566_1711del146	Benign (FN)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Proband counting (PS4_Supporting) Absent from population controls (PM2) *in silico* splicing models predict no effect on the native site (BP4)	y	3
CDH1 c.1711+2_1711+7delTAAGGG	r.1566_1711del146	Deleterious (TP)	LP	P	Null variant (Null variant (PVS1_Strong) RNA studies demonstrate abnormal splicing (PS3) Proband counting (PS4_Supporting) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		3
CDH1 c.1712-3T>G	r.1712_1720del9	Deleterious (TP)	VUS	VUS	RNA studies demonstrate abnormal splicing (PS3_Supporting) *in silico* splicing models predict a weakening of the native site (PP3)		n/a
CDH1 c.1766A>G (p.N589S)	WT	Deleterious (FP)	VUS	VUS	Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3) RNA studies demonstrate no abnormal splicing (BS3)		n/a
CDH1 c.2439+5_2439+8delGTAA	r.2388_2439del52	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Proband counting (PS4_Supporting) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	2
CDH1 c.2440-2A>G	r.2440_2449del10	Deleterious (TP)	LP	P	Null variant (Null variant (PVS1_Strong) RNA studies demonstrate abnormal splicing (PS3)		1

					Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)		
MLH1 c.116+5G>A	r.116+1_116+232ins227	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Located in a mutational hotspot (PM1) *in silico* splicing models predict a weakening of the native site (PP3)	y	1
MLH1 c.305A>C (p.E102A)	r.302_306del5	Benign (FN)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) Clinical phenotype is specific with disease (PP4_Moderate)	y	
MLH1 c.884+3A>G	r.791_884del94 r.791_1038del248	Deleterious (TP)	VUS	LP	Clinical phenotype is specific with disease (PP4_Strong) RNA studies demonstrate abnormal splicing (PS3) Located in a mutational hotspot (PM1) Absent from population controls (PM2) Co-segregation with disease (PP1_Moderate)	y	6
MLH1 c.2103+3A>G	r.1990_2103del114 r.1897_2103del207	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3) Clinical phenotype is specific with disease (PP4_Moderate)	y	3
MSH2 c.366+3A>G	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict no effect on the native site (BP4)		1
MSH2 c.645+3A>G	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) *in silico* splicing models predict no effect on the native site (BP4)		6
MSH2 c.2006-3T>G	r.2006_2210del205	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Clinical phenotype is specific with disease (PP4_Moderate) Absent from population controls (PM2) *in silico* splicing models predict a weakening of the native site (PP3)	y	1

MSH6 c.3231A>G (p.1077P)	WT	Benign (TN)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3)		3
MSH6 c.3417C>T (p.G1139G)	r.3416_3438del22	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) Clinical phenotype is specific with disease (PP4_Moderate) *in silico* splicing models predict a weakening of the native site (PP3)	y	1
MSH6 c.3801+5G>A	WT	Deleterious (FP)	VUS	LB	RNA studies demonstrate no abnormal splicing (BS3) Observed in healthy individuals (BS2)		33
MSH6 c.3802-7_3802-4delTCTT	r.3802_4001del200	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Co-segregation with disease (PP1) *in silico* splicing models predict a weakening of the native site (PP3) Clinical phenotype is specific with disease (PP4_Strong)	y	7
MSH2 c.1277-14C>G	r.1277-13_1277-1ins13 r.1277_1386del110	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) Clinical phenotype is specific with disease (PP4_Strong) *in silico* splicing models predict a weakening of the native site (PP3) Co-segregation with disease (PP1)	y	2
PMS2 c.803+5G>A	r.762_803del42 r.706_803del98	Deleterious (TP)	VUS	LP	RNA studies demonstrate abnormal splicing (PS3) Absent from population controls (PM2) Clinical phenotype is specific with disease (PP4_Moderate) *in silico* splicing models predict a weakening of the native site (PP3)	y	4

MSH2 c.942+3A>G	r.793_942del150	Deleterious (TP)	VUS	LP	Novel missense change where a different missense change is pathogenic (PM5) Absent from population controls (PM2) RNA studies demonstrate abnormal splicing (PS3) Clinical phenotype is specific with disease (PP4) *in silico* splicing models predict a weakening of the native site (PP3)	y	2