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Clinical exome sequencing vs. usual care for hereditary colorectal cancer diagnosis: A pilot comparative effectiveness study

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ABSTRACT

Background: Clinical exome sequencing (CES) provides the advantage of assessing genetic variation across the human exome compared to a traditional stepwise diagnostic approach or multi-gene panels. Comparative effectiveness research methods offer an approach to better understand the patient-centered and economic outcomes of CES.
Purpose: To evaluate CES compared to usual care (UC) in the diagnostic work-up of inherited colorectal cancer/polyposis (CRCP) in a randomized controlled trial (RCT).
Methods: The primary outcome was clinical sensitivity for the diagnosis of inherited CRCP; secondary outcomes included psychosocial outcomes, family communication, and healthcare resource utilization. Participants were surveyed 2 and 4 weeks after results return and at 3-month intervals up to 1 year.
Results: Evolving outcome measures and standard of care presented critical challenges. The majority of participants in the UC arm received multi-gene panels [94.73%]. Rates of genetic findings supporting the diagnosis of hereditary CRCP were 7.5% [7/93] vs. 5.4% [5/93] in the CES and UC arms, respectively ($P = 0.28$). Differences in privacy concerns after receiving CRCP results were identified (0.88 in UC vs 0.38 in CES, $P = 0.05$); however, healthcare resource utilization, family communication and psychosocial outcomes were similar between the two arms. More participants with positive results (17.7%) intended to change their life insurance 1 month after the first return visit compared to participants returned a variant of uncertain significance (9.1%) or negative result (4.8%) ($P = 0.09$).
Conclusion: Our results suggest that CES provides similar clinical benefits to multi-gene panels in the diagnosis of hereditary CRCP.

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer in the United States with an overall 5-year survival rate of 64% [1]. Survival rates significantly increase with early diagnosis [1] and screening colonoscopy is a successful preventative intervention [2]. Hereditary colorectal cancer (CRC), the majority of which is caused by pathogenic variants in genes associated with Lynch syndrome, accounts for approximately 3% of all newly diagnosed CRC cases [3–5]. Evaluation for Lynch syndrome has historically involved a multistep process beginning with personal and family history screening, followed by tumor tissue testing for mismatch repair (MMR) protein expression of the *MLH1, MSH2, MSH6* and *PMS2* MMR genes using immunohistochemistry (IHC) and/or/ microsatellite instability (MSI) and, finally, sequential germline genetic testing to establish a diagnosis [6]. This approach is relatively costly, requires multiple points of contact with patients, which is burdensome for patients/providers, has limited clinical sensitivity [7], and takes weeks to months before potentially reaching a diagnosis. Evaluation for other, rarer inherited CRC conditions, such as Familial Adenomatous Polyposis, causes by pathogenic variants in the *APC* gene, or *MUTYH*-associated Polyposis, caused by pathogenic variants in the *MUTYH* gene, includes discussion of personal and family history and directed germline genetic testing.

Massively parallel sequencing (MPS) is a transformative technology that has been rapidly integrated into the practice of medicine [8,9]. This technology has supported the development of multi-gene sequencing panels which allow simultaneous, cost-effective testing of many genes associated with the clinical indication of interest. Multi-gene panel testing [10] is replacing the sequential diagnostic approach for hereditary CRCP in clinical practice, though both are currently considered the standard diagnostic approach for hereditary CRCP evaluation. Multi-gene panels need to be continually updated and re-validated with the discovery of additional disease-associated genes. Patients with negative results on one cancer gene panel might benefit from being tested again in the future with a different or updated panel that includes additional, often newly identified, associated genes.

Clinical exome sequencing (CES) involves sequencing all genes in the exome and enables return of both diagnostic findings as well as medically actionable findings that are unrelated to the initial indication, or secondary findings (SFs). CES is increasingly being adopted by clinical laboratories to diagnose genetic diseases, aid treatment decisions and provide prognostic information [11–16]. However, the impacts of CES on clinical diagnosis, patient-centered outcomes and economic outcomes are not clear, and are potentially challenging to evaluate.

Comparative effectiveness research (CER) generates and synthesizes evidence through comparisons of alternative methods for healthcare. It can help patients, clinicians and health policy makers to make informed decisions that will improve healthcare at both the individual and population levels. The objective of our study was to assess the clinically actionable findings identified by CES compared to UC (usual care (UC) in the context of evaluation for inherited CRCP, and to explore the effects of CES on psychosocial outcomes, family communication and healthcare resource utilization using CER methods.

2. Materials and methods

2.1. Study design

We conducted a randomized controlled trial (RCT) to evaluate the comparative effectiveness of CES to UC in participants referred to medical genetics for evaluation for hereditary CRCP. The study design has been described in detail previously [17]. Eligible participants were unrelated adult patients referred to the University of Washington Medical Center (UWMC) Genetic Medicine Clinic, the Seattle Cancer Care Alliance (SCCA), or Kaiser Permanente Washington (formerly Group Health Cooperative) for genetic counseling for hereditary CRCP. All three of these institutions serve patients in King County in Washington State. The UWMC and SCCA also serve patients from the Washington, Wyoming, Alaska, Montana and Idaho (WWAMI) region. Most patients receiving care at these institutions have access to either private or federally funded health insurance. Informed consent of all participants was obtained and participant protection was assured under the University of Washington institutional review board.

A personal and/or family history of CRCP was the usual referral indication in these clinic populations. We excluded patients for whom single gene testing was indicated due to 1) a personal and/or family history consistent with a specific CRCP syndrome or 2) abnormal MMR protein expression from IHC testing suggesting a pathogenic variant in a single gene underlying their condition. After clinical UC genetic testing was ordered, informed consent and enrollment took place, and clinical and research blood volumes were drawn for all participants. Participants were then randomized to the UC or CES arm; participants in the CES arm also received UC genetic testing, after discussion with the University of Washington Institutional Review Board (IRB), to ensure that the research intervention of CES was not inferior to UC.

2.2. Usual care

Based on participants' CRCP related personal and family history, and clinician preference, two types of UC were offered: 1) tumor tissue testing for CRCP-related MMR gene protein expression with IHC and/or MSI and 2) genetic testing panels with variable numbers of hereditary CRCP-associated genes using MPS technology (BROCA [18], ColoSeq [19], and others). Clinical laboratories added genes to panels as new gene-disease association evidence was published; thus, the number of genes included on UC genetic testing panels was dynamic ranging from less than 10 to over 50. Fig. A.1 presents the expansion of the genes included on the ColoSeq panel over the course of the study. UC results were restricted to findings from only the UC clinical test ordered for the patient at the time of enrollment. Some UC genetic testing panels also included genes associated with other hereditary cancer conditions (such as *BRCA1/2* associated with hereditary breast and ovarian cancer). Clinicians ordered larger “pan-cancer” gene panels when indicated based on patient personal and/or family history. Thus, variants in non-CRCP hereditary cancer genes were returned to some participants from UC genetic testing panels in this study. We refer to variants detected by the UC approach that are not related to CRCP as non-CRCP diagnostic findings.

2.3. Clinical exome sequencing

All CES was conducted at the University of Washington, Northwest Clinical Genomics Laboratory (NCGL). Variants in a panel of hereditary CRCP genes were annotated. This hereditary CRCP gene panel included genes with a well-established association with hereditary CRCP and emerging hereditary CRCP genes. The number of CRCP-related genes annotated increased over the course of the study with the addition of genes with new evidence of association with hereditary CRCP. The original CES CRCP-related gene list included the following genes: *APC, BMPR1A, EPCAM, GREM1, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SCG5, SMAD4, STK11, TP53, CDH1, FLCN, PTCH1, TGFBR2, MLH3, PMS1* and *RET*. The following genes were added to the CES CRCP-related gene list during the study period: *POLD1, POLE, ATM, GALNT12, NBN, NTHL1, RINT1, RPS20, XRCC2, RNF43, LRP6, PTPN12, EMR3* and *AXIN2*.

Variants in a dynamic set of medically actionable, adult onset genes were also annotated as SFs from CES. This SFs gene list included the 55 genes associated with adult onset conditions on the American College of Medical Genetics and Genomics (ACMG) 59 actionable gene-disease pair list [13]. A description of the content and development of this SFs list, as well as the included and excluded genes, has been published

elsewhere [20,21]. Briefly, a formal return of results committee consisting of 14 practicing medical geneticists and 2 genetic counselors, as well as non-genetics providers, bioethicists and molecular laboratory representatives met regularly over the course of the study to develop and maintain the SFs gene list. Genes were included after unanimous agreement that they met the committee's definition of actionability, which included the existence of specific, defined medical recommendations leading to significant, tangibly improved outcomes in terms of morbidity and mortality after their implementation. Genes associated with pediatric onset conditions and genes that did not meet the actionability threshold for inclusion were excluded. CRCP-related genes were excluded from our SFs gene list. Variants in both CRCP-related and SF genes were interpreted based on the ACMG guidelines for the interpretation of sequence variants [22] or our internal approach prior to the ACMG publication [23].

Select Clinical Pharmacogenetics Implementation Consortium (CPIC) recognized functional pharmacogenomic variants and pathogenic variants in genes associated with carrier status were also annotated. The decision to annotate carrier status genes for return was based on participant interest and to increase the number of participants receiving a positive test result. Carrier status genes were chosen primarily based on the frequency of the condition in European ancestry populations (the ancestry of most participants) and pharmacogenetic variants were chosen based on a high level of evidence of an established relationship with drug metabolism and the ability of CES to identify the variant (Table A.1). The number of pharmacogenomic variants annotated increased slightly over the study period as additional variant-drug metabolism relationships were evaluated by the study team. Of note, only a subset of participants underwent testing for carrier status genes, subsequent to focus groups determining interest in them.

Only variants in annotated genes were evaluated and interpreted for return to participants. Pathogenic (P) variants, likely pathogenic (LP) variants and variants of uncertain significance (VUS) were reported in CRCP associated genes, but only pathogenic (P) variants were reported in the adult onset, medically actionable SF and carrier status genes. The decision not to return LP variants in medically actionable SF genes was made prior to the publication of the ACMG recommendations which advocate for doing so, and was based on an attempt to minimize the potential harms associated with returning variants with a lower prior probability of causing disease in this research cohort.

2.4. Return of results

Participants in both arms received their UC diagnostic results either by phone or in person at a first return visit. Participants in the CES arm were additionally returned CRCP-related results from CES at this visit. Approximately 2 months later at the second return visit, medically actionable SFs, carrier status results and pharmacogenetic variants were returned to participants in the CES arm. Participants in the UC arm received further review and discussion of family history during this second return visit. A licensed, certified genetic counselor, often accompanied by a medical geneticist, conducted the return of UC and CES results visits.

2.5. Healthcare resource utilization

Participant surveys for healthcare resource utilization and participant behavior data were developed de novo for this study and administered at the initial visit either online or by postal mail, 1 month after the first return visit, and then at 1, 4, 7, and 10 months after the second return visit. Participants responded to questions about their use of medical services, such as the number of visits to a genetic counselor and having CRCP or non-CRCP related medical procedures, as well as actual or intended changes to their life and health insurance. Survey instruments also asked participants how many first-degree family members they shared their genetic testing results with since their last visit. The instrument did not ask specifically about actions taken in response to participants receiving their genetic test results; we relied on differences between the study arms to estimate attribution.

2.6. Psychosocial outcomes

Participants in both arms also completed the FACToR (Feelings About genomiC Testing Results) questionnaire, the Veterans RAND 12 Item Health Survey (VR-12) [24], the Generalized Anxiety Disorder 7-Item Scale (GAD-7) [25], the Brief Patient Health Questionnaire Mood Scale (PHQ-9) [26], and a five-item version of the Mental Health Inventory (MHI-5) [27] to measure psychosocial impacts of testing results. These instruments were completed 2 weeks after the first return visit and 2 weeks, and 4, 7, and 10 months after the second return visit. The development and validation of the FACToR questionnaire has been described elsewhere [28]. In brief, the FACToR questionnaire was created from open-ended qualitative interviews with patients based on categories from the Multidimensional Impact of Cancer Risk Assessment (MICRA) questionnaire and additional categories suggested by interview findings [29]. The FACToR questionnaire has four subscales measuring participants' positive feelings (score range: 0–16), negative emotions (score range: 0–12), uncertainty (score range: 0–12) and privacy concerns (score range: 0–8) towards receiving genetic test results. For items that measure positive feelings, scores were reversed before being summed into a total score. Higher scores on each subscale indicate greater functional impairment.

2.7. Statistical analysis

We used descriptive statistics (frequencies, means, standard deviation (SD), proportions) to summarize participants' demographic characteristics, CRCP history, and types of UC testing. With comparable UC received in both arms, we assumed a higher detection rate of CRCP P/LP variants in the CES arm due to the additional sequencing of CRCP related genes anticipated when the trial was originally designed. Thus, our pre-specified primary test was a one-sided standard two-sample test comparing the proportions of participants identified with CRCP P/LP variants in both arms. We also performed standard two sided two-sample tests of proportions for health resources utilization outcomes, and two-sided standard *t*-tests for mean scores of psychosocial outcomes between the two randomization arms. We did not compare results from the surveys to medical records because the majority of the patients also received care outside of our healthcare system. While collection of insurance claims data for individual patients was considered, this approach was not considered practical given these were secondary outcomes. For exploratory descriptive analyses of healthcare resource utilization and psychosocial impact, we stratified groups of participants with different primary CRCP genetic testing results (P/LP [positive] vs. VUS vs. Negative [absence of P, LP, or VUS variants]). We used a Chi-square statistic and Wald-based 95% confidence intervals (CIs) for a trend analysis for proportions. The statistical significance level was set at $\alpha = 0.05$. All statistical analyses were conducted in Stata 14 software (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

3. Results

3.1. Participants

Fig. A.2 shows participant enrollment and study events throughout the project. From March 2012 to October 2016, 190 eligible participants were recruited and randomized to the UC and CES arms at the baseline visit. The majority of participants (179/190) were enrolled from the genetics clinics at the UWMC and SCCA. Expected UC testing was not performed for four participants (2 CES; 2 UC) after study enrollment, thus these participants were excluded from comparisons after

the baseline visit. One hundred and seventy-five participants attended the first return visit for their diagnostic genetic testing results (88 CES, 87 UC). Sixty-nine participants in the CES arm attended a second return visit and received medically actionable SFs results, carrier status results and pharmacogenetic variant results, and 71 participants in the UC arm had further review of family history at this time.

Sociodemographic characteristics, participant CRCP personal and family history, and the type of UC test received by randomization status are presented in Table 1. The participant mean age was 52.9 years (SD:13.1), and 51.6% were female. Participants in the UC and CES arm were similar in their mean age, gender distribution, marital and employment status, education level, household income, and household size. Thirty-one (36.84%) participants in the UC arm had a personal history of CRC compared to 26 (33.68%) in the CES arm. The University of Washington ColoSeq™ hereditary CRC gene panel accounted for just over half of the UC tests conducted in both arms (53.68% vs 53.68%); notably, as described above, the number of genes included on ColoSeq™ increased several times over the course of the study (Fig. A.1).

3.2. Hereditary cancer diagnostic results

Approximately half of the participants who received diagnostic hereditary cancer results did so over the phone (92/175, 52.6%). The other half received these results in person (83/175, 47.3%). Five (5.4%) participants in the UC arm and seven (7.5%) participants in the CES arm had a P or LP CRCP-related variant identified ($P = 0.28$, Table 2). Six of these 12 participants had a personal history of colorectal cancer. One CES participant had two pathogenic variants in *MUTYH*. Homozygosity or compound heterozygosity for two pathogenic variants in *MUTYH* is associated with autosomal recessive *MUTYH* associated polyposis and CRC. Fifteen (16.1%) participants in the UC arm and 21 (22.6%) in the CES arm had a CRCP-related VUS identified ($P = 0.13$). Participants having CES testing also had UC testing, as noted above. For the CRCP genes tested by both approaches, there were no differences in the P/LP results returned to those participants. One participant in the CES arm had a VUS returned from UC that was not returned from CES. Five participants in the UC arm and three participants in the CES arm had a P variant in a non-CRCP related hereditary cancer gene returned at their first return visit. An additional two participants (one in the UC arm and one in the CES arm) had a VUS in a non-CRCP hereditary cancer related gene identified by their UC testing. The number and interpretation of CRCP and non-CRCP hereditary cancer related variants are shown by randomization arm in Table A2. Combining all CRCP-related variants in both arms, eight P/LP variants in Lynch syndrome related genes and seven P/LP variants in non-Lynch CRCP related genes were returned.

3.3. Return of medically actionable SFs, carrier status and pharmacogenetic variants

One participant (1.45%) in the CES arm was returned a P variant in the *BRCA1* gene, which is associated with hereditary breast and ovarian cancer, at the second return visit as a medically actionable SF. A second participant in the CES arm had a *BRCA2* P variant identified by CES, but this participant did not return for a second visit. This participant had also received a UC test that reported this *BRCA2* P variant; thus, it had been returned by his clinical providers at his first return visit. The majority of participants in the CES arm (60/69, 87%) had one or more pharmacogenomic variant returned, and 7 of the 23 participants (30%) who had consented for return of carrier status results had a P variant returned in one of these genes.

3.4. Healthcare resources utilization

One hundred and twenty-three participants (59 in the UC arm, 64 in the CES arm) responded to the healthcare resources utilization survey administered 1 month after the first return visit. Selected healthcare resource utilization was similar between the UC vs. CES arms (Table 3). These analyses were not corrected for multiple comparisons. Healthcare resources included visits to medical providers, medical procedures and changes to health and life insurance. In terms of family communication, 14% more participants in the UC arm have shared their genetic test results with their first-degree family members than the CES arm ($P = 0.06$). The non-responders did not differ significantly from the responders regarding their baseline characteristics and results status (results not shown here).

3.5. Psychosocial outcomes

As shown in Table 4, the scores for the negative emotion, positive feelings, and uncertainty subscales on the FACToR survey were not statistically different by randomization arms 2 weeks after the receipt of CRCP-related test results. A small difference in the privacy concerns subscale scores was seen at this time point (0.88 in UC vs 0.38 in CES, $P = 0.05$). However, this pattern did not persist at later visits. No statistically significant differences in any of the FACToR subscale scores between arms were seen in surveys administered at subsequent time

Table 1
Participant characteristics by randomization arm at baseline[a].

	UC ($N = 95$)	CES ($N = 95$)
Demographics		
Annual household income > $100,000	40.00%	36.84%
Mean age, years (SD)	51.8 (14.0)	53.4 (12.5)
College & graduate degree	55.79%	57.90%
Female	56.84%	46.32%
Employed	58.95%	71.58%
Now married	63.16%	69.47%
Household size ≥ 2	78.95%	86.32%
Race (self-reported)		
Hawaiian/other pacific islander	1.53%	0.00%
Black/African American	0.00%	1.53%
Asian	2.11%	8.42%
American Indian/Alaska Native	10.53%	8.42%
White	84.2%	81.1%
CRCP history		
CRC diagnosis	36.84%	33.68%
Colon Polyps	86.32%	80.00%
UC testing received[b]		
None	2.11%	2.11%
MSI and/or IHC	3.16%	5.26%
BROCA™ panel[c]	18.95%	21.05%
Commercial panel[d]	22.11%	21.05%
ColoSeq™[e]	53.68%	53.68%

[a] No statistically significant differences for any characteristics between the two arms.

[b] Numbers might not add up to 100% because some participants received more than one type of UC.

[c] The BROCA™ pan cancer gene panel included the following genes at the start of the study: *APC, ATM, ATR, BABAM1, BAP1, BARD1, BMPR1A, BRCC36, BRIP1, CDH1, CDK4, CDKN2A, CHEK1, CHEK2, EPCAM, FAM175A, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PRSS1, PTEN, RAD50, RAD51B, RAD51C, RAD51D, RBBP8, RET, SMAD4, STK11, TP53, TP53BP1, UIMC1, VHL, XRCC2,* and *XRCC3*. The following genes were added to the BROCA™ panel during the course of the study: *AKT1, AXIN2, BRCA1, BRCA2, CTNNA1, FANCM, FH, FLCN, GALNT12, GEN1, GREM1, HOXB13, MEN1, MET, MITF, NF1, NTHL1, PALLD, PDGFRA, PIK3CA, POLD1, POLE POT1, PRKAR1A, PTCH1, RB1, RECQL, RINT1, RPS20, SDHB, SDHC, SDHD, SLX4* and *SMARCA4*. The following genes were removed from the BROCA™ panel during the course of the study: *BABAM1, BRCC36, RAD50, RBBP8, STK11, TP53BP1, UIMC1 and XRCC3.*

[d] Other panels included colon cancer and pan-cancer panels from GeneDx ($N = 35$), Myriad Genetics ($N = 2$), Ambry Genetics ($N = 1$) and Invitae ($N = 1$).

[e] See Fig. A.1 for genes included on the ColoSeq™ panel throughout the course of the study.

Table 2
Number of participants with diagnostic results in hereditary cancer genes by randomization arm.

	UC (*N*/%)	CES (*N*/%)	*P*-value[d]
	Total = 93	Total = 93	
CRCP related			
P/LP variant(s)	5 (5.4%)	7[a] (7.5%)	0.28
VUS(s)	15 (16.1%)	21[b] (22.6%)	0.13
Non-CRCP[c] related			
P/LP variant(s)	5 (5.4%)	3 (3.2%)	0.77
VUS(s)	1 (1.1%)	1 (1.1%)	0.50

[a] One CES participant was compound heterozygous for 2 CRCP related P variants in the *MUTYH* gene.
[b] One CES participant had three CRCP related VUS findings (two in *APC* and one in *AXIN2*); one CES participants had two CRCP related VUS findings (one in *APC*, one in *PTCH1*); one CES participant had one CRCP related P variant (*MSH2*) and one CRCP related VUS finding (*PMS2*).
[c] Non-CRCP related findings were due to non-CRCP genes on the UC multi-gene panels, not considered as SFs.
[d] *P*-value based on one-sided two-sample test of proportions.

Table 3
Participant healthcare resource utilization 1 month after the first return visit by randomization arm.

In the past month…	Randomization arms		*P*-value[a]
	UC (*N* = 59)	CES (*N* = 64)	
	n (%)	*n* (%)	
No visits to a specialist/doctor	40 (67.8%)	36 (56.3%)	0.19
No visits to a genetic counselor	39 (66.1%)	48 (75.0%)	0.28
Had any medical procedures	15 (25.4%)	19 (29.7%)	0.59
Had any evaluation of cancer	12 (20.35)	19 (29.7%)	0.23
Have made changes to health insurance	3 (5.1%)	4 (6.3%)	0.77
Have made changes to life insurance	2 (3.4%)	4 (6.3%)	0.46
Have shared genetic test results with 1st-degree blood family member (s)	50 (84.7%)	45 (70.3%)	0.06

[a] *P*-value based on two-sided two-sample test of proportions.

points (Table 5). The mean total scores on VR-12, GAD-7, PHQ-9 and MHI-5 administered 2 weeks after the second return visit were also similar (Table 6). Analyses of psychosocial outcomes were also not corrected for multiple comparisons.

3.6. Exploratory analyses by CRCP results (P/LP vs. VUS vs. negative)

We conducted exploratory stratified analyses of healthcare resource utilization and psychosocial outcome across groups of participants with different primary CRCP genetic testing results (P/LP vs. VUS vs. Negative).

During the month after the first return visit, similar percentages of participants with each type of CRCP result had shared their CRCP-related results with at least one first-degree blood relative (Table A.3). There was no significant difference in the number of participants who made changes to their life insurance based on the type of result returned ($P = 0.13$). A higher proportion of participants with positive (P/LP) results (17.7%) had thought about changing their life insurance 1 month after the first return visit compared to participants returned a VUS (9.1%) or negative result (4.8%); however, this observed trend was not statistically significant ($P = 0.09$). Participants were not asked what specific type of insurance changes they thought about making. There were no other trends for other aspects of healthcare resource utilization based on type of CRCP-related result returned at this time point, including thoughts about or actual changes to health insurance or the average number of visits to a specialist doctor or genetic counselor. No obvious trends were seen at 1, 4 or 7 months after the second return visit.

The overall mean scores on the FACToR negative emotion subscale were relatively low. Participants with positive (P/LP) CRCP-related results had a higher mean score on the FACToR negative emotion subscale (1.56) than participants returned VUS (0.60) or negative (0.57) results 2 weeks after the first return visit; however, this observed trend was not statistically significant ($P = 0.27$; Table A.4). We observed the same pattern on the uncertainty, and privacy concern subscales at this time point as well. Participants with positive (P/LP) CRCP-related results had lower scores on the positive emotion subscale than those returned other types of results. There were no differences seen on the FACToR subscales 2 weeks, 4 and 10 months after the second return visit (Table A.4). No differences were seen on the other psychosocial instruments based on type of variant returned. (Table A.5).

4. Discussion

We compared the clinically actionable findings from UC vs. CES in patients having clinical genetic testing for hereditary CRCP, and found similar proportions of participants with CRCP related P/LP variants and VUS in both arms. The implementation of CES in this context did not significantly increase the number of VUS returned to participants, which has been cited in the literature as a concern with expanded gene panel testing [30]. VUS rates from multi-gene panels and CES will depend on the number of genes annotated, variant interpretation criteria and lab specific policies for return. The CES approach does not currently appear to provide additional immediate diagnostic value in this context beyond multi-gene cancer panel tests. CES does allow future reinterpretation of genes newly associated with CRCP that were not on the UC panel test.

One participant was returned a P variant identified by CES in a gene (*BRCA1*) associated with an adult onset, medically actionable condition. An additional participant had a *BRCA2* P variant identified on CES, but did not return to receive this result from the study team. This rate of medically actionable SFs identified is consistent with previous estimates [20,23,31].

The current National Comprehensive Cancer Network (NCCN) 2017 guidelines support universal screening of newly diagnosed CRC patients with IHC or MSI testing before gene-specific germline testing. The guideline also recommends multi-gene panel testing be offered in the context of genetic expertise and counseling, among selected populations such as those from familial, high-risk clinic-based populations or with early-onset CRC [6,32,33]. Recent studies have explored using multi-gene panels to identify variants of interest in cancer predisposition genes beyond those evaluated through IHC or MSI [34,35]. There are currently no recommendations for if or when to consider expanded or alternative multi-gene panel tests for patients being evaluated for hereditary cancer, and best practice recommendations for clinical laboratory policies on data reanalysis are needed [36]. Approximately half of the CRCP-related P/LP variants returned in this study were not

Table 4
FACToR subscale scores 2 weeks after the first return visit by randomization arm.

Subscale	UC (*n* = 59)	CES (*n* = 64)	*P*-value[a]
	Mean (SD)	Mean (SD)	
Negative emotion, 0–12	0.56 (0.93)	0.84 (1.81)	0.28
Positive feelings, 0–16	7.20 (4.60)	6.45 (3.92)	0.33
Uncertainty, 0–12	1.92 (2.14)	1.97 (2.50)	0.90
Privacy concerns, 0–8	0.88 (1.81)	0.38 (0.93)	0.05

[a] *P*-values were based on two-sided *t*-test for two-sample comparison of means.

Table 5
FACToR subscale scores 2 weeks, 4 and 10 months after the second research return visit (RRV2) by randomization arm.

FACToR	UC	CES	*P*-value[a]
	Mean (SD)	Mean (SD)	
2 weeks after RRV2	(*n* = 47)	(*n* = 48)	
Negative emotion, 0–12	0.28 (0.71)	0.40 (1.11)	0.53
Positive feelings, 0–16	7.81 (4.57)	7.13 (4.27)	0.42
Uncertainty, 0–12	1.09 (1.90)	1.13 (1.94)	0.92
Privacy concerns, 0–8	0.47 (1.23)	0.69 (1.60)	0.46
4 months after RRV2	(*n* = 57)	(*n* = 63)	
Negative emotion, 0–12	0.40 (1.03)	0.41 (0.99)	0.96
Positive feelings, 0–16	10.14 (4.832)	9.60 (4.52)	0.53
Uncertainty, 0–12	1.44 (2.31)	1.46 (2.12)	0.96
Privacy concerns, 0–8	0.53 (1.23)	0.29 (0.77)	0.19
10 months after RRV2	(*n* = 51)	(*n* = 52)	
Negative emotion, 0–12	0.16 (0.54)	0.19 (0.56)	0.75
Positive feelings, 0–16	10.29 (5.06)	9.37 (5.16)	0.35
Uncertainty, 0–12	1.18 (1.85)	1.15 (2.24)	0.96
Privacy concerns, 0–8	0.47 (1.39)	0.25 (0.68)	0.31

[a] *P*-values were based on two-sided t-test for two-sample comparison of means.

Table 6
Results of other psychosocial scales 2 weeks after the second return visit by randomization arms.

Other instruments	UC	CES	*P*-value[a]
	Mean (SD)	Mean (SD)	
VR-12	(*n* = 62)	(*n* = 63)	
Physical component score	45.47 (12.50)	47.31 (11.83)	0.39
Mental component score	50.13 (10.00)	50.11 (9.07)	0.99
GAD-7 total score	(*n* = 62)	(*n* = 62)	
	3.34 (4.12)	3.02 (4.11)	0.66
PHQ-9 total score	(*n* = 61)	(*n* = 63)	
	4.11 (4.29)	3.71 (4.55)	0.62
MHI-5 total score	(*n* = 62)	(*n* = 63)	
	76.69 (16.79)	77.86 (17.22)	0.70

[a] *P*-values were based on two-sided t-test for two-sample comparison of means.

in Lynch syndrome related genes, providing further evidence to support the consideration of larger multi-gene panels, rather than Lynch panels, as a first-line test in the evaluation for hereditary CRCP.

Concern regarding the potential psychological burden of pursuing and receiving results from genomic sequencing tests has been cited in the literature, as have questions regarding the downstream costs to healthcare systems of incorporating this testing into clinical practice [37–40]. In this study, we did not observe differences in psychosocial impacts, healthcare use or family communication outcomes between the UC and CES arms, or based on type of CRCP-related result returned. These findings are consistent with a similar study exploring genome sequencing in 100 healthy adults in a primary care setting that found no additional participant anxiety or depression related to genome sequencing when compared with standard family history assessment [41].

5. Limitations

Due to the small sample size and the similarity in the number of CRCP-related results returned across arms, our study was likely underpowered to evaluate the impact of different CRCP findings on psychosocial outcomes, family communication and healthcare utilization. It is also possible that no difference in healthcare use was found because participants in both arms were already following a high risk cancer prevention screening program prior to testing or had not yet accessed healthcare resources when the surveys were administered. The increased use of multi-gene panels for UC clinical testing, and the increasing number of CRCP-related genes included on these panels throughout the time period of the study, led to few expected differences in primary findings between the two arms. Our study also included relatively few participants from underserved or ethnically and sociodemographically diverse populations so may not be generalizable across all patient care settings. Standardized outcome measures for psychosocial consequences after genetic tests are still evolving and the reliability and validity of the FACToR needs to be re-confirmed among different populations with different clinical conditions. More studies evaluating the clinical significance of elevated scores of FACToR are also warranted. Finally, participant healthcare resource utilization questions were not specific to actions taken in response to receiving the test results, thus making attribution difficult given the relatively small sample size and number of findings returned. We recommend future studies inquire specifically about actions taken in response to test results, even though such attribution may be challenging for participants.

6. Conclusions

CES and UC testing, consisting primarily of gene panels, had similar rates of P/LP variants and VUS returned in patients being evaluated for hereditary CRCP. Evolving clinical practice of increasing the number of genes on the UC panel to include all associated CRCP genes presented a major challenge for the original comparative effectiveness design in this study. CES in the evaluation for hereditary CRCP currently does not add diagnostic value beyond UC testing at the time of the initial test, though the potential for reanalysis of newly associated genes is a noted benefit of CES. The current standard practice of MSI/IHC with subsequent germline testing or multi-gene panels is appropriate for hereditary CRCP evaluation until further evidence is available regarding the benefits of exome level data in this context. Future studies with larger sample sizes, that explore phenotypes for which there is a higher proportion of unknown genetic etiology and that include diverse and underserved patient populations to evaluate the psychosocial, familial communication and economic outcomes of CES using standardized, validated measures are needed.

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Conflict of interest disclosure

All authors declare no relevant financial interests in this manuscript.

Appendix A. Appendix

Table A.1
Select pharmacogenetic variants and carrier status genes analyzed by CES at conclusion of the trial.[a]

	Association
Pharmacogenetic variants	
*CYP2C19*2, *3*	Impaired responsiveness to Clopidogrel
*CYP2C9*2, *3,*4,*5,*6,*9,*11*	Warfarin sensitivity
*CYP4F2*3*	Warfarin resistance
*DPYD*2A, c.496A > G/M166V*	Dihydropyrimidine dehydrogenase deficiency
*SCLO1B1*5*	Statin induced myopathy
*TPMT*2,*3A,*3B,*3C,*4*	6-mercaptopurine sensitivity; Azathioprine sensitivity
Carrier status genes	
ACADM	Medium Chain Acyl-CoA Dehydrogenase Deficiency
BCHE	Pseudocholinesterase Deficiency
CFTR	Cystic Fibrosis
G6PC	Glycogen Storage Disease, Type 1A
GBA	Gaucher disease
GJB2	GJB2-related non-syndromic hearing loss
HBB	Beta thalassemia
HEXA	Tay-Sachs disease
PAH	Phenylalanine Hydroxylase Deficiency
SERPINA1	Alpha-1 Antitrypsin deficiency

[a] The pharmacogenetic variants list was dynamic throughout the course of the study and not all participants randomized to CES had carrier status genes annotated.

Table A2
Number and interpretation of variants identified by UC and CES diagnostic testing and returned to participants in each randomization arm.

	Randomization arm			
	UC		CES	
	P/LP	VUS	P/LP	VUS
Lynch syndrome related				
MLH1	0	0	0	1
MSH2	2	2	2	2
MSH6	0	1	0	1
PMS2	3	2	1	3
Total	5	5	3	7
Non-Lynch syndrome related				
APC	1	5	0	7
AXIN2	0	1	1	1
MUTYH	1	1	3	0
PMS1	0	0	0	2
POLE	0	0	0	2
PTCH1	0	0	0	1
SEMA4A	0	0	0	2
SMAD4	0	0	0	2
STK11	0	1	0	0
TP53	0	0	1	0
XRCC2	0	0	0	1
CDH1	0	1	0	1
Total	2	9	5	19
Non-CRCP related[a]				
BRCA1	1	0	1	0
BRCA2	0	0	1	0
CHEK2	2	0	1	0
HOXB13	2	0	0	0
PALB2	0	0	0	1
Total	5	0	3	1

[a] Non-CRCP findings at the first return visit in both arms were due to non-CRCP genes included on the UC multi-gene panels.

Table A.3
Participant healthcare resource utilization 1 month after the first return visit by types of results returned.

In the past month	Genetic testing results[a]		
	Positive (P/LP)	VUS	Negative
	N = 17 (%)	*N* = 25 (%)	*N* = 84 (%)
Number of visits to a specialist doctor			
0	13 (74.5)	14 (63.6)	49 (58.3)
1	2 (11.8)	6 (27.3)	21 (25.0)
2	1 (5.9)	1 (4.6)	6 (7.1)
3	1 (5.9)	0 (0)	8 (9.5)
Missing	0 (0)	1 (4.6)	0 (0)
Number of visits to a genetic counselor			
0	14 (82.4)	15 (68.2)	58 (69.1)
1	3 (17.7)	5 (22.7)	23 (27.4)
2	0 (0)	0 (0)	2 (2.4)
3	0 (0)	1 (4.6)	0 (0)
Missing	0 (0)	1 (4.6)	1 (1.2)
Had any medical procedures			
No	14 (82.4)	18 (84.8)	56 (66.7)
Yes	3 (17.7)	4 (18.2)	27 (32.1)
Missing	0 (0)	0 (0)	1 (1.2)
Had any evaluation of cancer			
No	15 (88.2)	17 (77.3)	60 (71.4)
Yes	2 (11.8)	5 (22.7)	24 (28.6)
Health insurance changes			
Have made	0 (0)	2 (9.1)	5 (6.0)
Intend to	3 (17.7)	1 (4.6)	4 (4.8)
Thought about	0 (0)	2 (9.1)	3 (3.6)
Have not	14 (82.4)	17 (77.3)	72 (85.7)
Life insurance changes			
Have made	0 (0)	0 (0)	6 (7.1)
Intend to	0 (0)	1 (4.6)	2 (2.4)
Thought about	3 (17.7)	2 (9.1)	4 (4.8)
Have not	14 (82.4)	19 (86.4)	72 (85.7)
Shared genetic test results with 1st-degree blood family members			
No	3 (17.7)	5 (22.7)	20 (23.8)
Yes	14 (82.4)	17 (77.3)	64 (76.2)

[a] Genetic testing results included both CRCP and non-CRCP findings.

Table A.4
FACToR subscale scores at each time point by type of result returned.

	Positive (P/LP)	VUS	Negative
	Mean (SD)	Mean (SD)	Mean (SD)
2 weeks after first return visit	(*n* = 16)	(*n* = 25)	(*n* = 82)
Negative Emotion, 0–12	1.56 (2.56)	0.60 (1.32)	0.57 (1.14)
Positive Feelings, 0–16	9.88 (3.76)	7.60 (4.04)	5.98 (4.13)
Uncertainty, 0–12	3.19 (3.12)	2.16 (2.49)	1.63 (2.01)
Privacy Concerns, 0–8	0.88 (1.75)	0.56 (1.76)	0.59 (1.28)
2 weeks after second return visit	(*n* = 11)	(*n* = 14)	(*n* = 70)
Negative Emotion, 0–12	0.91 (1.45)	0.14 (0.36)	0.29 (0.89)
Positive Feelings, 0–16	8.18 (2.79)	8.07 (4.73)	7.23 (4.58)
Uncertainty, 0–12	1.18 (1.99)	1.00 (1.96)	1.11 (1.92)
Privacy Concerns, 0–8	0.55 (1.29)	0.14 (0.53)	0.67 (1.56)
4 months after second return visit	(*n* = 13)	(*n* = 20)	(*n* = 87)
Negative Emotion, 0–12	1.00 (1.35)	0.65 (1.18)	0.26 (0.89)
Positive Feelings, 0–16	9.77 (2.35)	11.35 (4.42)	9.53 (4.93)
Uncertainty, 0–12	2.77 (2.74)	2.10 (2.71)	1.10 (1.89)
Privacy Concerns, 0–8	0.23 (0.83)	0.30 (0.80)	0.45 (1.08)
10 months after second return visit	(*n* = 12)	(*n* = 15)	(*n* = 76)
Negative emotion, 0–12	0.33 (0.65)	0.53 (1.06)	0.08 (0.32)
Positive feelings, 0–16	10.33 (3.23)	10.00 (5.72)	9.71 (5.27)
Uncertainty, 0–12	1.00 (1.41)	2.40 (3.22)	0.95 (1.77)
Privacy concerns, 0–8	0.08 (0.29)	0.47 (0.99)	0.38 (1.19)

Table A.5
Results of other instruments by genetic finding groups at 2 weeks after the first return visit.

	Positive	VUS	Negative
	Mean (SD)	Mean (SD)	Mean (SD)
VR-12	(*n* = 16)	(*n* = 25)	(*n* = 84)
Physical component score	47.63 (12.46)	49.78 (9.48)	45.15 (12.70)
Mental component score	50.23 (8.78)	49.88 (9.00)	50.18 (9.87)
GAD-7 total score	(*n* = 16)	(*n* = 25)	(*n* = 83)
	2.94 (4.39)	2.88 (3.18)	3.31 (4.32)
PHQ-9 total score	(*n* = 16)	(*n* = 24)	(*n* = 84)
	3.88 (5.51)	3.46 (3.06)	4.05 (4.54)
MHI-5 total score	(*n* = 16)	(*n* = 25)	(*n* = 84)
	74.38 (19.05)	79.00 (13.99)	77.32 (17.45)

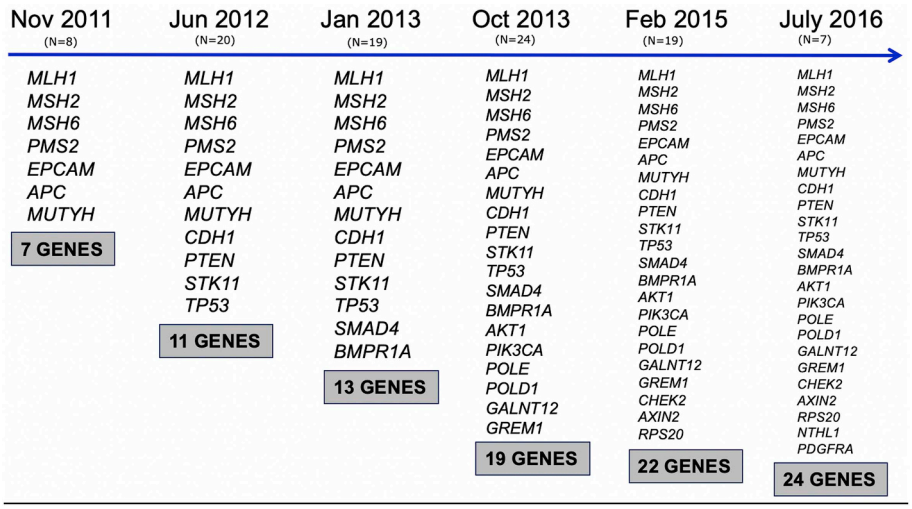

Fig. A.1. Genes on the University of Washington, Coloseq™ hereditary CRC panel over course of study and number of participants who received each version of the panel.

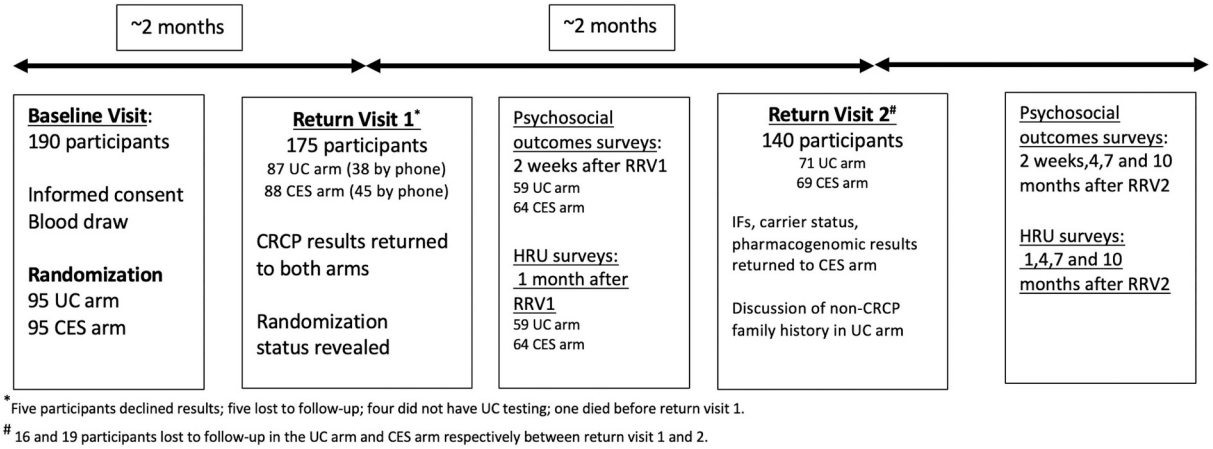

Fig. A.2. Study flow & participant enrollment diagram.

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