Carrier testing for hereditary colorectal cancer in British Columbia and Yukon

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Abstract

***Background*:** Cascade carrier testing for hereditary cancer enables identification of individuals most likely to benefit from intensive screening and preventive measures. Despite predicted positive health outcomes, uptake of carrier testing for hereditary colorectal cancer syndromes has been shown as relatively low. We report rates of familial testing in a publicly-funded hereditary cancer clinic in Canada.

***Methods*:** Testing uptake and demographic factors between 1997 and 2016 were assessed for families in which the index patient received testing through the provincial Hereditary Cancer Program (HCP) for British Columbia (BC) and the Yukon. Analyses were conducted for syndromes with an increased risk for colorectal cancer, including Lynch syndrome (*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*), Familial Adenomatous Polyposis (*APC*), and additional moderate to high penetrance genes (*STK11*, *TP53*, *SMAD4*, *MUTYH*, *PTEN*, and *CHEK2*). Descriptive statistics were utilized and all analyses were two-tailed.

***Results*:** The mean age at carrier testing was 41.2 years. The median time between disclosure of index and carrier test results was 8.3 months, with 61% of carrier tests in females. Among eligible first-degree relatives, 31% (267/851) underwent carrier testing. The cascade carrier rate was calculated to be 1.56 carrier tests per index case. Of 67 cancer diagnoses in carriers, 63% were diagnosed prior to carrier testing.

***Interpretation*:** A significant proportion of individuals at-risk for hereditary colorectal cancer do not undergo carrier testing. This highlights the need to explore barriers to testing, consideration of interventions to promote uptake, and more aggressive efforts by hereditary cancer programs to reach this highest risk population.

Introduction

It is estimated that approximately 5 – 6% of colorectal cancers are due to a well-established hereditary cancer syndrome.[1,2] Genetic testing in at-risk relatives, known as cascade carrier testing, has the potential to identify those most likely to benefit from increased screening and prophylactic measures, thereby leading to earlier detection and decreased cancer incidence.[3] Additionally, the cost-effectiveness of broad panel genetic testing relies on uptake of targeted carrier testing of at-risk relatives, and arguably the effectiveness of hereditary cancer programs in general.[4-6] The most common hereditary colorectal cancer syndrome, Lynch syndrome is inherited in an autosomal dominant manner and accounts for up to 5% of colorectal cancers.[2] Lynch syndrome has up to an 80% and 60% risk for colorectal and endometrial cancer, respectively.[7] In more rare hereditary colorectal cancer syndromes such as Familial Adenomatous Polyposis (FAP) and biallelic *MUTYH,* without appropriate surveillance there is up to a 100% colorectal cancer risk.[8] Given the significantly higher cancer risk among these syndromes compared to the general population, identifying those at risk is an important health care priority. Lynch syndrome has been given special designation by the Centers for Disease Control in the United States as a Tier 1 condition to address carrier testing due to its impact on public health.[9]

Reports suggest variable carrier testing rates in clinic-and cancer registry-based studies of at-risk relatives, ranging from 34% - 75%.[4, 10-23] Most individuals who have received carrier testing for Lynch syndrome are satisfied with receiving testing in the long-term,[11] as carrier testing enables more informed decision-making and personalized healthcare.[24] Non-carriers also benefit from carrier testing, as negative test results may reduce cancer-related anxiety [25] and relieve non-carriers from intensive screening.[16] Many studies have found a significantly higher

number of females than males receiving carrier testing.[12, 16, 21] Some studies have also related age to carrier testing uptake, with lower rates of testing in first-degree relatives (FDRs) below age 25 for Lynch syndrome [21] and above age 40 for FAP.[16] To understand the uptake of carrier testing in a Canadian context, we reviewed 20 years of carrier testing uptake for hereditary cancer syndromes associated with an increased risk for colorectal cancer in the population-based Hereditary Cancer Program (HCP) that is the sole provider of publicly-funded cancer genetic testing across BC and the Yukon.

Methods

Subjects

Research Ethics Board approvals from the University of British Columbia and the University of the Fraser Valley were obtained. Demographic data, and personal medical and family history information for patients assessed between January 1, 1997 and December 31, 2016 were obtained using HCP clinical and BC Cancer electronic chart databases. We retrospectively assessed carrier testing uptake in families where the first family member found to carry a pathogenic or likely pathogenic variant, known as the index patient, was identified through our program. For adult-onset syndromes, relatives were considered eligible for carrier testing if they were 19 years of age or older, alive, and living in BC or the Yukon. In the case of childhood onset syndromes, living relatives of all ages were eligible. The autosomal dominant conditions Lynch syndrome (*MLH1, MSH2, MSH6, PMS2*, and *EPCAM*), FAP (*APC*), Li-Fraumeni Syndrome (*TP53*), Peutz-Jeghers Syndrome (*STK11*), Juvenile Polyposis (*SMAD4*), and PTEN-hamartoma/Cowden Syndrome (*PTEN*), and *CHEK2* where FDRs of gene positive individuals have a 50% risk of carrying the familial pathogenic variant were included.[16] The autosomal recessive syndrome MUTYH-associated polyposis was also included, where at-risk

relatives may have a 25% risk of homozygous/biallelic *MUTYH* pathogenic variant status.[16] Only families identified through index cases with biallelic *MUTYH* pathogenic or likely pathogenic variants were included in the analysis, with only siblings deemed as eligible individuals for testing.

Cascade Carrier Analysis

In-depth pedigree analyses were performed for each index patient to ascertain the number of eligible FDRs and the number of relatives tested by degree (first through fourth degree). In the case of multiple tests performed in one family, the first individual tested was considered the index patient. The time interval between the index and carrier tests was determined by calculating the difference between the date of index and carrier test result disclosures.

Demographic Factors and Cancer History

Summary statistics were calculated to describe the population of index and carrier tests by age, gender, rural or urban residence, and referral method. The types and number of cancers diagnosed in individuals before and after carrier testing were assessed to determine the health impact of carrier testing. All cancer types were included in this analysis, including non-melanomatous skin cancers and cervical cancer.

Statistical Analysis

Descriptive statistics were calculated using Microsoft Excel 2016 and were reported as mean and standard deviation or median and range for continuous variables, and proportion or frequency for categorical variables. R Environment for Statistical Computing version 3.3.4 was used to perform Chi-squared tests to determine uptake by gender and age, as well as univariate and multivariate analyses to assess the relationship of age, gender, urban/rural residence, and cancer diagnosis with testing uptake. Univariate and multivariate analyses were employed to

assess factors related to the time interval between index and carrier tests. All statistical analyses were two-tailed with a statistical significance of $p \leq 0.05$.

Results

Study Cohort

The study cohort included 245 index patients tested between January 1, 1997 and December 31, 2016. During this period, 382 carrier tests were performed for the relatives of the index patients. Demographic data and source of referral for index and carrier tests are reported in Table 1. For the index patients, this included 150 (61%) females and 95 (39%) males. Pathogenic or likely pathogenic variants were identified in Lynch syndrome genes for the majority of individuals, at 64% (157/245). The mean age at index testing was 49.3 ± 15.1 years, with 85% (209/245) and 10% (24/245) of patients located in urban and rural residences, respectively (Table 1). Among those undergoing carrier testing, 61% (233/382) were female and 39% (149/382) were male. The mean age at carrier testing was 41.2 ± 17.7 years. The majority of individuals undergoing carrier testing were living in urban regions (87%; n=332) and 9% (n=35) in rural regions, and 4% unspecified (n=15). The most common source of referral was a medical specialist for the index patients (54%), and by self-referral for those undergoing carrier testing (48%). Self-referral was the only characteristic of the index patient that significantly correlated with increased carrier testing in FDRs (p=0.034).

Cascade Carrier Testing Analysis

Figure 1 shows the uptake of carrier testing in eligible FDRs of in-province index patients. Of 851 eligible FDRs, 267 (31%) received carrier testing. The highest uptake was 50% of FDRs for *CHEK2* (5/10, 95% CI 24–76%) and *SMAD4* (3/6, 95% CI 19–81%), and the lowest uptake was 5% of FDRs of biallelic *MUTYH* (3/58, 95% CI 1-15%). Figure 2 shows the cascade

carrier ratios (the number of carrier tests per index test) for each gene. These include all known carrier tests (first through fourth degree relatives) performed for relatives of in-province positive index patients. The highest cascade carrier testing rate was obtained for *SMAD4*, with 2.50 relatives per index test (5/2). *PMS2* had the lowest rate at 0.60 carrier tests per index (6/10). The total cascade carrier rate across all genes was 1.56 carrier tests per index (382/245).

Notably, a significant number of eligible family members had not undergone carrier testing. The frequency of families with FDRs tested to the frequency of families with the same number of FDRs eligible are compared in Figure 3. We identified 91 families with eligible, in-province FDRs with no carrier tests performed.

The median time to carrier testing was 8.3 months (range: 0 to 170.3 months). The median time to testing for the five Lynch syndrome genes was 8.2 months (range: 0 to 143.0 months). Time to testing and range by gene are reported in Table 2. Age of the patient significantly correlated to the time to uptake of carrier testing, with younger family members having a longer time gap before pursuing testing ($p<0.001$). Among relatives who were female ($p=0.090$), and who self-referred themselves to the program ($p=0.077$), a trend towards shorter time to uptake for testing was observed.

Figure 4 depicts the proportion by FDRs tested with respect to time from index diagnosis. A plateau for familial testing appears to be reached in 2-3 years for *MUTYH* and other rare genes. However, for *APC* and Lynch syndrome, there continues to be an increasing number of family members accessing testing after 6 years.

Cancer Diagnoses

A total of 67 cancer diagnoses were identified in positive carriers for a colorectal cancer-related gene. The majority (48%) of the diagnoses were colorectal cancer (32/67), followed by

breast cancer (n = 8), and endometrial cancer (n = 7). The remaining diagnoses included non-melanomatous skin cancer and cervical cancer (n = 3 for both); lymphoma, ovarian cancer, and prostate cancer (n=2 per cancer type), and one case each of brain cancer, jejunum cancer, leukemia, lung cancer, pancreatic cancer, thyroid cancer, cancer of the tongue, and sarcoma. The majority (63% ; 42/67) of cancer diagnoses occurred before carrier testing, and 37% (25/67) were diagnosed after testing. The distribution of genes with a cancer diagnosis before genetic testing were *MLH1* (n=16), *MSH2* (n=13), *APC* (n = 4), and (n = 3) for each *MSH6, TP53,* and *STK11*.

Interpretation

In this retrospective analysis, we assessed the uptake of genetic testing in 245 families with a known familial pathogenic or likely pathogenic variant in a colorectal cancer-related gene. Across the 12 genes analyzed, the uptake of testing in eligible FDRs was 31%. Unsurprisingly, the most common genes tested for were those related to the relatively common Lynch syndrome which comprised 64% of index diagnoses; overall uptake was highest in female relatives. Previous reports of uptake of testing for Lynch syndrome in FDRs has ranged between 34-94% [14-16, 20-22].

To date, reports on cascade carrier rates have differed widely across studies. We identified a rate of 1.64 carrier tests per index for Lynch syndrome families, and 1.56 across all 12 genes. Similar rates of 1.5 [14] and 1.04 [13] relatives tested per index patient have previously been reported for Lynch syndrome. However, reported rates of 4.6[26] and 3.6[16] carrier tests per index reveal large variability in cascade carrier testing between populations. This emphasizes the value of a population-based approach to assessing carrier testing, as uptake rates may differ greatly between clinics and countries. Whereas carrier testing for MAP and other rare syndromes

seems to plateau earlier on (2-3 years), we see a longer trajectory for *APC* and Lynch syndrome, still increasing after 6 years. Other studies have documented intervals ranging from 2 years [16] to 12 years [21] for family members with Lynch syndrome to seek testing before a plateau was reached.

Demographically, the proportion of patients from rural areas in this study was lower than what we might expect based on census data. From 1997-2016, 9% of patients completing carrier testing for hereditary colorectal cancer were from rural regions in British Columbia; this is lower than the overall rural population of the province being 18% in 1996 [27]and 14% in 2016 [28]. Some studies have shown disparities in awareness in rural populations [29] whereas others have demonstrated higher uptake of testing when using alternative methods like telephone counseling.[30]These studies support the development and use of alternative modes for education and genetic service delivery to rural populations to ensure equitable access.

A previous cancer diagnosis has been identified as a predictor of carrier testing.[31] We found that 63% of cancer diagnoses in positive carriers were diagnosed before carrier testing, similar to a percentage of 63.4% from another Canadian study on Lynch syndrome. [32] - Considering that a major goal of carrier testing is to prevent hereditary cancer through intensive screening and prophylactic measures, it is important to reach at-risk relatives before cancer diagnoses.[33] Further investigation into the health impact of carrier testing for colorectal-cancer related syndromes is necessary. We identified 91 families seen at HCP with eligible, in-province FDRs who have had no carrier tests performed. Males in particular were less likely to undergo carrier testing as compared to females. For Lynch syndrome, some studies have found higher testing rates among females [13, 16] while others did not report a gender difference.[10, 11]

Future Directions

Fear of life insurance and mortgage implications is a commonly reported reason for declining carrier testing.[18] In 2017, Canada enacted the Genetic Non-Discrimination Act, preventing insurance companies, employers, and those involved in any contract from requiring individuals to disclose genetic test results.[34] In late 2018 the Quebec Court of Appeal ruled several parts of the law to be outside of the jurisdiction of the federal government, *ultra vires,* which was appealed by the Canadian Coalition for Genetic Fairness. As a results, the case is expected to be heard by the Supreme Court of Canada. [35,36,] Research investigating carrier testing rates before and after enactment will be an important subject of future research. Recent systematic reviews on cascade carrier screening point to the limitation of the index patient being responsible to inform all at-risk relatives.[9, 23] Although standard procedure in most hereditary cancer programs, including that of BC Cancer, studies where health professionals also contacted relatives have reported higher rates of testing uptake. [15, 16, 20-22] Policy and legal considerations are needed when proposing direct contact with relatives, making this an area for further research. Our results highlight the need to explore barriers to testing and develop tailored interventions to promote testing uptake. This will help guide decision-making regarding education and resource allocation in order to optimize carrier testing uptake.

Limitations

An important limitation to this study is that we included only in-province index tests and associated carrier tests in the analyses in order to ensure accuracy of data. However, many carrier tests have been performed through the HCP for family members with the initial family member testing positive residing outside of British Columbia, and these families were not included in our study. It is possible that some at-risk relatives had received out-of-province carrier testing for

which we did not have records. Therefore, the cascade carrier testing rate may be higher than determined. Additionally, cancer diagnoses included only those for in-province positive carriers. due to limited access to data for out-of-province family members' cancer diagnoses. Although our data captured index and carriers tests over a period of 20 years, individuals tested between 2017 and the time of data collection were not included in the analyses. Future analyses that assess carrier testing post-2016 will serve as valuable contributions to the growing body of carrier testing literature.

Conclusion

In this study, we identified that a significant proportion of individuals at-risk for hereditary colorectal cancer in BC and the Yukon have not received carrier testing. Studies that explore barriers to testing in this particular population may elucidate possible avenues for interventions to promote testing uptake, with the ultimate goals of early detection and prevention of hereditary cancer. Sub-optimal rates for cascade carrier testing highlight the need to identify barriers and develop strategies to improve genetic testing rates in this highest risk population.

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Table 1: Demographic characteristics of individuals who have received in-province index or carrier testing through the Hereditary Cancer Program (HCP)

	Total (%)	Mean age ± SD
Index tests	245 (100)	49.3 ± 15.1
Gender		
Female	150 (61)	—
Male	95 (39)	—
Residence		
Urban	209 (85)	—
Rural	24 (10)	—
Unspecified	12 (5)	—
Referral Source		
Self	16 (7)	—
Family Doctor	46 (19)	—
Specialist Doctor	129 (54)	—

Family Member	8 (3)	—
HCP	1 (0.4)	—
Unspecified/Other	23 (10)	—
Carrier tests	382	41.2 ± 17.7
Gender		
Female	233 (61)	42.2 ± 16.9
Male	149 (39)	39.4 ± 18.8
Residence		
Urban	332 (87)	—
Rural	35 (9)	—
Unspecified	15 (4)	—
Referral Source		
Self	185 (48)	—
Family Doctor	77 (20)	—

Specialist Doctor	37 (10)	—
Family Member	33 (9)	—
HCP	5 (1)	—
Unspecified/Other	45 (12)	—

Table 2: Time difference between index and carrier tests reported in months

Gene	**Median time difference (months)**	**Range (months)**	**Mean age of carrier tests ± SD**	**Proportion Females**
PTEN	8.0	4.6 - 27.2	31.1 ± 19.6	68.8
MLH1	7.8	0 - 91.3	41.1 ± 15.7	58.2
MSH2	11.9	0 - 143	43.9 ± 14.7	61.2
MSH6	6.0	1.1 - 43.5	46.8 ± 19.1	58.3
PMS2	11.6	2.9 - 13.4	56.0 ± 8.8	66.7
EPCAM	3.2	3.2 - 29.8	43.0 ± 23.0	0
MUTYH (biallelic index)	6.1	4.8 - 21.3	53.0 ± 11.0	83.3
APC	12.9	1.4 - 170.3	33.6 ± 20.3	65.5
STK11	8.4	0 - 36.4	48.0 ± 24.0	57.1
SMAD4	24.16	6.0 - 28.7	32.0 ± 28.0	20.0
CHEK2	3.7	2.7 - 25.1	46.0 ± 17.0	100
TP53	7.6	1.1 - 84.7	35.6 ± 17.8	64.3

TOTAL	**8.3**	**0 - 170.3**	**41.2 ± 17.7**	**61.0**
Lynch Total	**8.2**	**0 - 143.0**	**43.4 ± 15.9**	**58.9**

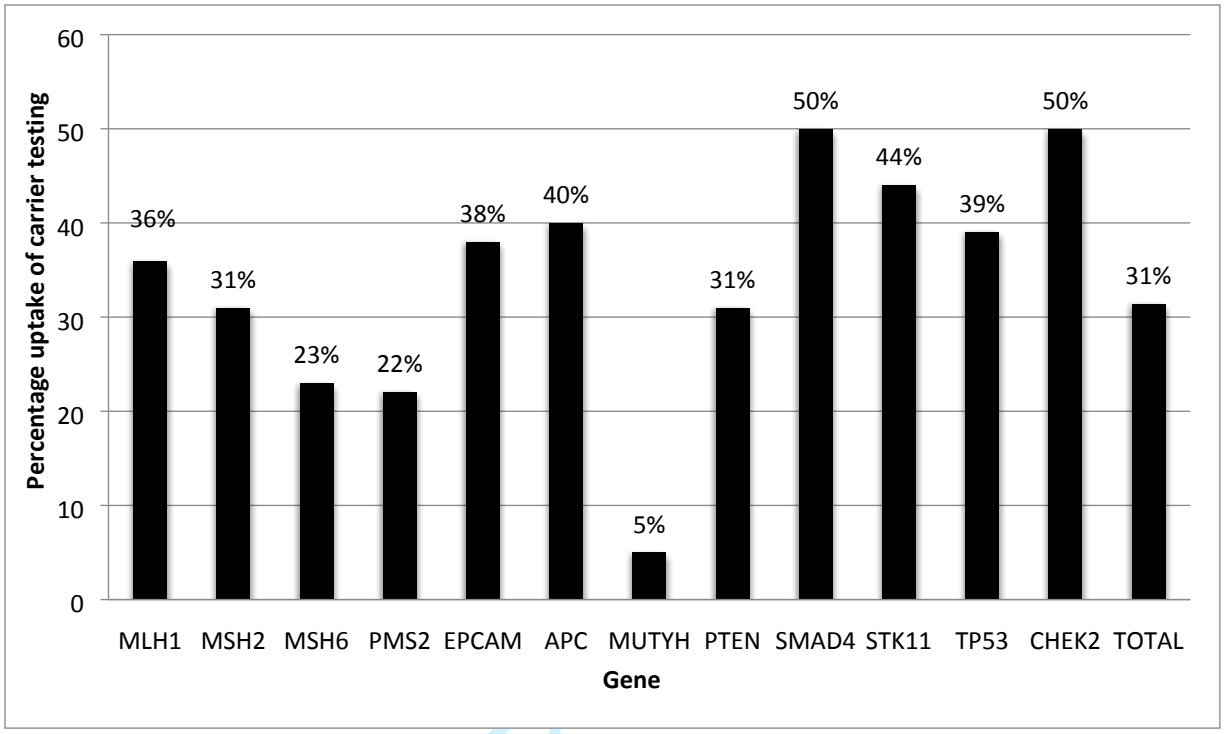

Figure 1: Percentage of eligible FDRs who have received carrier testing. Carrier testing for FDRs has been performed in 36% (79/220, 95% CI 30–42%) for *MLH1*, 31% (51/167, 95% CI 24–38%) for *MSH2*, 23% (27/116, 95% CI 16–32%) for *MSH6*, 22% (8/36, 95% CI 11–38%)) for *PMS2*, 38% (3/8, 95% CI 13–70%) for *EPCAM*, 40% (46/115, 95% CI 32–49%)for *APC*, 5% (3/58, 95% CI 1-15%) for *MUTYH* (biallelic), 31% (14/45, 95% CI 19–46%) for *PTEN*, 50% (3/6, 95% CI 19–81%) for *SMAD4*, 44% (8/18, 95% CI 25–66%) for STK11, 39% (20/52, 95% CI 26–52%) for *TP53*, and 50% (5/10, 95% CI 24–76%) for *CHEK2*. The combined carrier testing uptake in FDRs across all genes is 31% (269/851, 95% CI 29–35%).

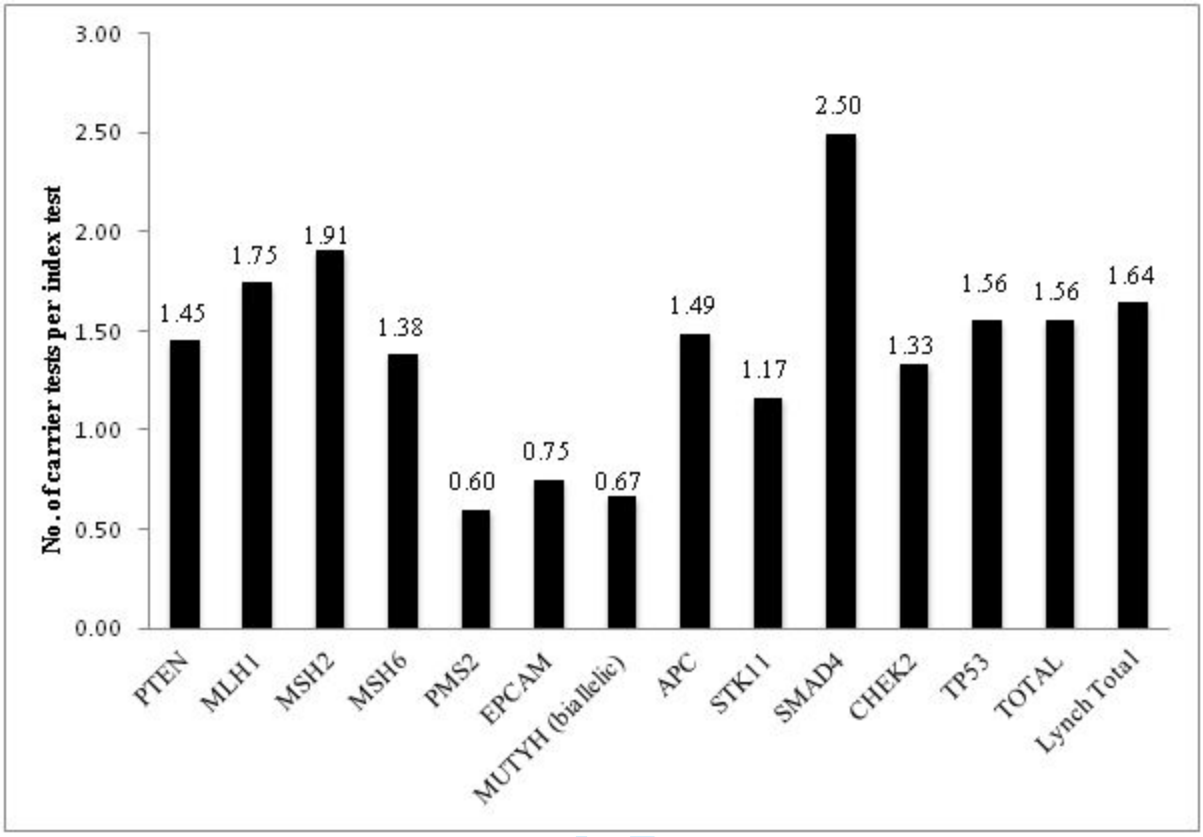

Figure 2: Cascade carrier testing ratios (carrier tests/index tests) by gene. Cascade carrier testing rates of 1.45 (16/11) for *PTEN*, 1.75 (110/63) for *MLH1*, 1.91 (103/54) for *MSH2*, 1.38 (36/26) for *MSH6*, 0.60 (6/10) for *PMS2*, 0.75 (3/4) for *EPCAM*, 0.67 (6/9) for *MUTYH*, 1.49 (58/39) for *APC*, 1.17 (7/6) for *STK11*, 2.50 (5/2) for *SMAD4*, 1.33 (4/3) for *CHEK2*, and 1.56 (28/18) for *TP53* were obtained. The total cascade carrier rate across genes was 1.56 (382/245), and 1.64 (258/157) for the five Lynch syndrome genes.

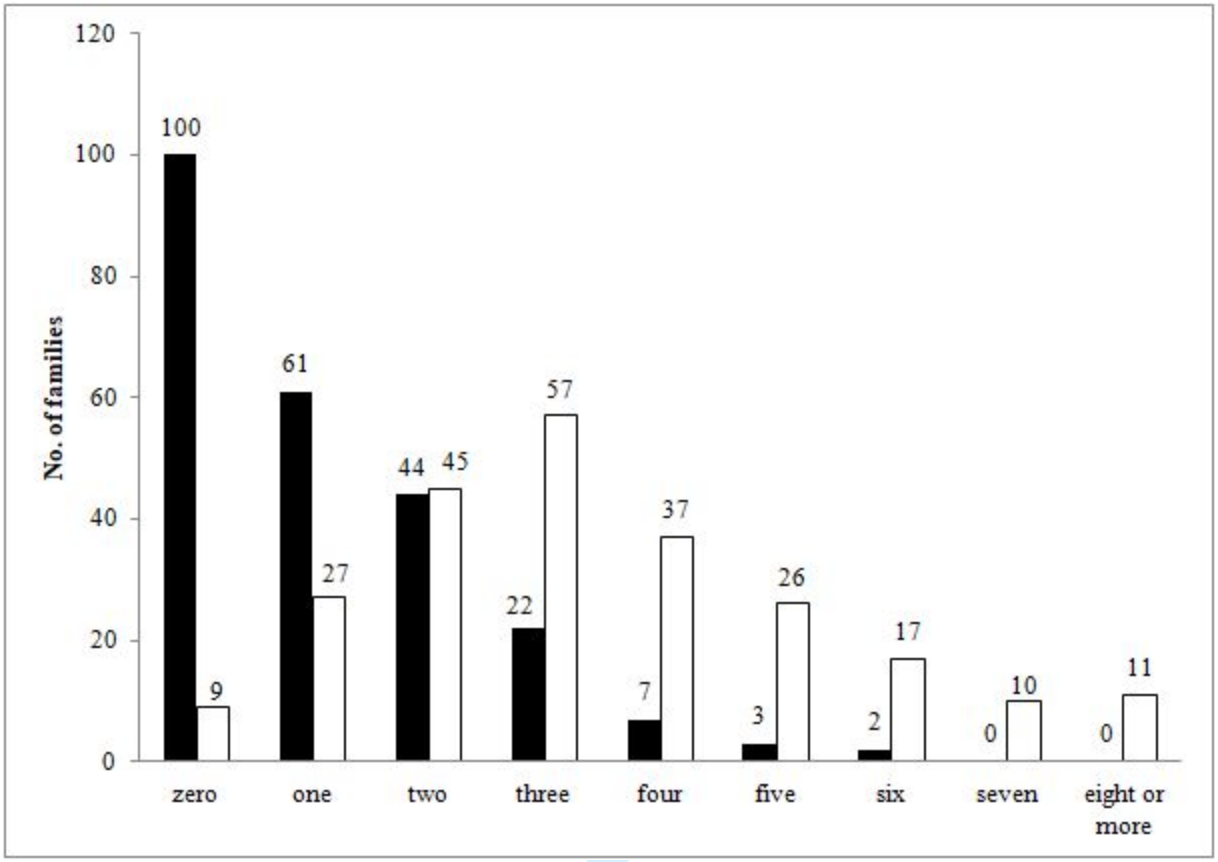

Figure 3: Side-by-side comparisons of the frequency of families with FDRs tested to the frequency of families with that same number of FDRs eligible. There are 91 families with eligible, in-province FDRs who have had zero carrier tests performed (the difference between FDR tested and FDR eligible in the zero column); the number actually tested designated with black bars, and the total eligible designated with white bars

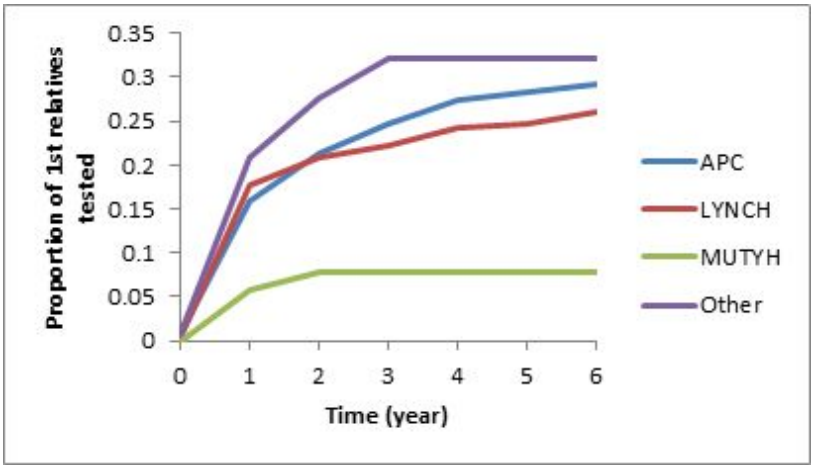

Figure 4: Proportion of FDRs who have received carrier testing with respect to time from index testing diagnosis. The blue line represents carrier testing in *APC* (index cases = 39), the red line Lynch syndrome (index cases = 157), the green line biallelic *MUTYH* (index cases = 9), and the purple line represents carrier testing for the remaining five colorectal cancer-related syndromes analyzed (*STK11*, *SMAD4*, *PTEN*, *CHEK2*, and *TP53)* (index cases = 40).

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	6-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Fig.1

		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-9
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.