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Lynch syndrome: the influence of environmental factors on extracolonic cancer risk in *h*MLH1 c.C1528T mutation carriers and their mutation-negative sisters

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Abstract Lynch Syndrome (LS) is a cancer susceptibility syndrome caused mostly by mutations in the mismatch repair genes, *h*MLH1, *h*MSH2 and *h*MSH6. Mutation carriers are at risk of colorectal and endometrial cancer and, less frequently, cancer of the ovaries, stomach, small bowel, hepatobiliary tract, ureter, renal pelvis and brain. The influence of environmental factors on extracolonic cancer risk in LS patients has not been investigated thus far. The aim of this study was to investigate some of these factors in South African females carrying the *h*MLH1 c.C1528T mutation and their mutation-negative relatives. Data were collected from 87 mutation-positive females and 121 mutation-negative female relatives regarding age, cancer history, hormonal contraceptive use, parity, duration of breast feeding, height, weight and age at first birth, last birth, menarche and menopause. Influence of these factors on cancer risk was analysed by mixed-effects generalised linear models. Extracolonic cancer occurred in 14% (12/87) of mutation-positive females versus 7% (8/121) of mutation-negative females, ($P = 0.0279$, adjusted for age and relatedness between women). Breast cancer was the most common extracolonic cancer. An association was found for oral contraceptive use and extracolonic cancer risk in mutation-negative females only. No association was found for any of the other risk factors investigated, when adjusted for age. This might be due to the scarcity of extracolonic cancers in our data. Future knowledge on the influence of additional environmental factors on cancer risk in LS females can lead to evidence-based lifestyle advice for mutation carriers, thereby complementing the prevention strategies available today. In addition, it can contribute to an integrated model of cancer aetiology. Therefore, this study should be taken as a thrust for further research.

Keywords Lynch syndrome · Extracolonic cancers · Environmental factors

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Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome (LS) is a cancer susceptibility syndrome caused mostly by mutations in the mismatch repair genes,

*h*MLH1, *h*MSH2 and *h*MSH6. Colorectal and endometrial cancers are the cancer types most frequently reported; mutation carriers are at an estimated lifetime risk of up to 70 and 27–71%, respectively [1, 12, 23, 33, 34]. In addition, cancers of the ovaries, stomach, small bowel, hepatobiliary tract, ureter, renal pelvis and brain are part of the HNPCC tumour spectrum [35, 38].

Surveillance programs in LS patients aiming at colorectal cancer are highly effective [17, 30]. Yet, the available screening methods for gynaecologic cancer are unreliable [11, 27, 28, 32]. Prevention programs aiming to modify environmental risk factors have not been established thus far, very likely because such factors have not been adequately identified.

In LS, expression of disease is very heterogeneous. This phenotypic heterogeneity has been attributed to genetic and allelic heterogeneity and the influence of sex, genetic modifiers and environmental factors [10, 13, 14, 16, 18, 24, 33, 37]. Investigations regarding the influence of environmental factors on phenotypic expression in LS mutation carriers have been limited to colorectal cancer risk only, and the cohorts investigated were generally small [10, 19, 36, 37].

In South Africa, a relatively large 'homogeneous' cohort predisposed to LS has been described previously, consisting of 15 families carrying the *h*MLH1 c.C1528T mutation [26, 30, 31]. Prior research in this cohort has investigated the occurrence of extracolonic cancers and the influence of modifier genetic factors on age of cancer onset and cancer diagnosis [3, 13]. Notably, breast cancer was the most common extracolonic cancer in the cohort.

In the general population, several environmental risk factors are known to affect breast cancer risk. Early age at menarche, late age at menopause, postmenopausal obesity, high age at first birth, low age at last birth and oral contraceptive use are positively associated, whereas long duration of breastfeeding, premenopausal obesity and high parity are inversely associated [15]. The risk of endometrial and ovarian cancer is raised by nulliparity, early menarche, late menopause and obesity, whereas oral contraceptive use has a protective effect on ovarian and endometrial cancer risk. Other factors have been associated with these cancers but show inconclusive results, including short duration of breastfeeding, older age at first birth and young age at last birth [2, 9].

As it was assumed that breast, endometrial and ovarian cancers would be the most common extracolonic cancers in our cohort, we investigated the influence of environmental factors known to be important in these cancer types in females carrying the *h*MLH1 c.C1528T mutation.

A recent study on mutation-negative first degree family members of BRCA-mutation carriers, showed them to be at an elevated risk for breast cancer when compared to the general population, suggesting an important influence of environmental and/or genetic modifying factors in mutation carriers and non carriers [29]. In order to test whether the previously listed environmental factors were of particular importance in mutation-negative females, we assessed their influence on cancer risk in mutation-negative female relatives of the *h*MLH1 c.C1528T mutation carriers. This has the additional advantage that the influence of the environmental factors on extracolonic cancer risk in mutation-positive females can be compared more reliably, as data on cancer risk and the influence of environmental factors in South African females lack reliability. These may differ from females in the western world, due to genetic and environmental differences and the influence of gene-environment interactions.

Aim

The aim of this study was to investigate the influence of environmental factors on extracolonic cancer risk in South African females and their mutation-negative relatives.

Materials and methods

In the 1980's a database was established of individuals with a diagnosis of colorectal cancer under the age of 50 by a collaboration of the Colorectal Surgery Unit at Groote Schuur Hospital and the Division of Human Genetics at the University of Cape Town, South Africa. To date, almost 500 probands and their family members have been registered. Efforts have been made to detect the disease causing mutations in these families. The c.C1528T mutation in the *h*MLH1 gene is the most common mutation detected and has so far been found in 15 families of mixed ancestry [26].

For the present study, all females known to be carriers of the *h*MLH1 c.C1528T mutation were selected from the database. Then, females related to these women who tested negative for the c.C1528T mutation were selected as a control group. At least two attempts were made to contact each of them between August and December 2006. Data on age, cancer history, parity and age at first and last birth were obtained in interviews using a structured questionnaire. Information on deceased women was provided by interviewing first-degree relatives. In addition, living participants were interviewed regarding their age of menarche, age of menopause, duration of breast feeding and hormonal contraceptive use. Height and weight were measured when possible (61% of cases).

Two families were excluded as they consisted of only male mutation-positive members, one family was excluded because we lost track of the only mutation-positive female

Table 1 Recruitment data

Test result	Mutation-positive individuals	Mutation-negative blood related individuals
Total number of individuals	94	199
Data available	87 (93%)	121 (61%)
Untraceable	7 (7%)	78 (39%)

Total number of females tested for the *h*MLH1 c.C1528T mutation: 317

Total number of related females tested for the *h*MLH1 c.C1528T mutation: 293

more than 10 years ago. 94 Mutation-positive and 199 mutation-negative females from the remaining 12 families were selected from the database. Of these, 14 mutation-positive and 9 mutation-negative females were deceased. 7 Mutation-positive women and 78 mutation-negative women were untraceable. The remaining 73 mutation-positive females and 112 mutation-negative living females were all willing to participate in the study. For the 23 deceased women, a first-degree relative was able to provide information on parity, age at first and last birth and the cause and date of death in all cases. Therefore, 87 mutation-positive females and 121 mutation-negative females were included in our analysis (Table 1).

The median time between data collection and cancer diagnosis was 8 years. Pathology reports and tumour tissue blocks were obtained where possible; this was the case in 65% of the cancers.

Ethics approval for the present study was obtained from the Research Ethics Committee of the University of Cape Town (REC/REF 466/2006). The majority of participants included in the study live in remote and socio-economically challenged areas of South Africa where the postal system is unreliable. As the collection of written consent of all participants was not feasible, we decided to collect only verbal consent. All living women and first degree relatives of deceased women provided verbal consent to participate in the study.

Statistical methods

For descriptive purposes, data was stratified by mutation status and the presence or absence of extracolonic cancer. These were summarised as number of women with non-missing data, and either mean and standard deviation, or median and range (for skewed data).

Given the small size of our cohort, the occurrence of any extracolonic cancer, rather than specific cancers, was analysed as primary outcome measure. As the women were closely related to one another, and their data thus not statistically independent, traditional statistical methods for independent dichotomous variables (Chi-squared, logistic regression) are not valid to assess the relevant associations. Mixed-effects generalised linear models, with a binomial family, and the default logit link were used, as they allow us to adjust for family-relationship (as random effect) and for other known confounders, such as age (as fixed effect). For all our comparisons, we modelled the risk of extra-colonic cancer as outcome, and adjusted for both age and family membership. That means all our tests are comparing those with extracolonic cancer to those without.

Our first analysis was to test for an association between extra-colonic cancer risk and mutation status. We then tested the association between extracolonic cancer risk and each of the factors listed in Table 3, separately in the mutation bearers and in those who do not bear that mutation. The extracolonic cancer odds ratios and their 95% confidence intervals are also given in Table 3.

Results

About 14% (12/87) of mutation-positive females reported at least one extracolonic cancer versus 7% (8/121) of mutation-negative females. After adjustment for age and family of origin, this difference was statistically significant with a *P*-value of 0.0279. Breast cancer was the most common extracolonic cancer reported in mutation-positive females, followed by endometrial cancer (Table 2).

Data on age at menarche, age at menopause, months of breastfeeding, contraceptive use and Body Mass Index (BMI) are summarized in Table 3. As these factors could only be investigated in living participants, the number of individuals and the number of extracolonic cancers in this group were small.

The factors summarised in Table 3 were all tested for an association with extracolonic cancer, and none were statistically significant in the mutation bearers. In the non-mutation bearers, having used contraceptives, multiplied the odds of ECC with 3.7, making it almost 4 times as likely to develop a tumour if one ever used contraceptives (compared to if one did not).

Table 2 Extra-colonic cancers in mutation-negative females

Mutation	Alive				Deceased				All	
	Positive		Negative		Positive		Negative			
	Number	% of *n*	Number	% of *n*	Number	% of *n*	Number	% of *n*	Number	% of *n*
Number, *n*	73		112		14		9		208	
Colorectal cancer	15	21	0	0	6	43	0	0	21	10
Breast cancer	4	5	2	2	3	21	2	22	11	5
Endometrial cancer	3	4	0	0	0	0	0	0	3	1
Ovarian cancer	1	1	0	0	0	0	0	0	1	0
Other cancer type	2	3	3	3	2	14	1	11	8	4
Any extracolonic cancer	7	10	5	4	5	36	3	33	20	10
Any cancer	20	27	5	4	10	71	3	33	38	18

Table 3 Descriptive statistics for investigated factors, as well as odds ratios for ECC for each of the factors

Investigated factor	Mutation-positive			Mutation-negative		
	ECC	NEC	OR (95% CI)	ECC	NEC	OR (95% CI)
Number, *n*	12	75		8	113	
Parity, median (range)[1]	4 (0–11)	2 (0–9)	1.1 (0.8–1.6)	3 (0–9)	2 (0–11)	1.0 (0.7–1.3)
Age at first birth, median (range)[2]	22 (17–25)	21 (16–41)	0.8 (0.7–1.1)	23 (20–24)	21 (16–42)	1.0 (0.7–1.2)
Age at last birth, median (range)$_3$	40 (23–44)	29 (17–44)	1.1 (0.9–1.3)	38 (20–45)	29 (17–48)	1.0 (0.9–1.1)
Age at menarche (years, mean ± SD)[4]	13.8 ± 1.6	14.2 ± 1.8	1.0 (0.5–1.8)	15.5 ± 2.4	14 ± 1.9	1.3 (1.0–1.7)
Age at menopause (years, mean ± SD)[5]	44.0 ± 6.6	44.1 ± 5.7	0.9 (0.7–1.2)	51.8 ± 3.5	45.2 ± 6.4	1.4 (1.0–2.0)
Duration of breastfeeding (months, mean ± SD)[6]	22.6 ± 14.3	20.1 ± 22.0	1.0 (0.9–1.1)	92.0 ± 80.9	30.3 ± 36.1	1.0 (1.0–1.0)
Contraceptive use (ever, number (%))[7]	3 (25%)	55 (73%)	0.2 (0.0–1.8)	3 (38%)	8 (7%)	3.7 (1.3–10.9)
BMI (kg/m^2, mean ± SD)[8]	34.0 ± 11.2	28.6 ± 6.9	1.0 (0.9–1.2)	31.4 ± 4.8	28.6 ± 8.1	1.1 (1.0–1.2)

For example, the odds ratio for parity, would be the odds of ECC versus NEC for each extra child. *ECC* extracolonic cancer. *NEC* no extracolonic cancer

[1] Data available for 87 mutation positive and 121 mutation negative females

[2] Data available for 87 mutation positive and 121 mutation negative females

[3] Data available for 80 mutation positive and 98 mutation negative females

[4] Data available for 71 mutation positive and 16 mutation negative females

[5] Data available for 55 mutation positive and 79 mutation negative females

[6] Data available for 32 mutation positive and 42 mutation negative females

[7] Data available for 41 mutation positive and 85 mutation negative females

[8] Data available for 29 mutation positive and 67 mutation negative females

Conclusion and discussion

This is the first study on the influence of environmental factors on extracolonic cancer risk in LS mutation carriers. We did not find a significant effect of any of the factors investigated, namely, parity, age at first and last birth, age at menarche and menopause, breastfeeding, contraceptive use and BMI, after adjustment for age. A plausible explanation is that the genetic influence of the LS-predisposing mutation greatly outweighs the influence of any environmental factor, especially as these cancers tend to occur at a relatively young age when people have been exposed to environmental factors for a relatively short period of time.

Modifying factors in cancer susceptibility syndromes

In contrast with our own findings, some previous studies on the influence of environmental factors on colorectal cancer risk in LS mutation carriers and cancer risk in other susceptibility syndromes detected significant associations.

With regards to LS, smoking has been associated with an increased risk of colorectal cancer risk [10, 37] whereas

fruit consumption, dietary fibre intake and the use of aspirin were found to be protective factors [6, 10]. However, studies on the influence of consumption of alcohol, meat and complex starch found no significant associations with colorectal adenoma formation or cancer risk [5, 36, 37]. With the exception of the study on the influence of aspirin and complex starch, these studies were small and investigated a different spectrum of environmental factors and cancers than assessed in this study.

More comprehensive research has been carried out in BRCA mutation carriers, who are at an elevated risk for breast and ovarian cancer. Previous research on the influence of specific environmental factors shows an important influence of obesity, alcohol use, animal fat consumption and physical inactivity [21]. Interestingly, the influence of other risk factors differs from their effect in the general population: age at menarche and menopause, important risk factors for breast cancer in the general population, do not significantly increase breast cancer risk in BRCA mutation carriers [7]. With regards to oral contraceptive use, an increase of breast cancer risk was seen for ever use and a longer duration of use before first pregnancy [4]. The latter is not seen in the general population, whereas a decrease of breast cancer risk in the 10 years after stopping is seen in the general population but not in BRCA1 and BRCA2 mutation carriers [4, 39]. We did not find an association between age at menarche, age at menopause or oral contraceptive use and risk of extracolonic cancer risk in mutation carriers, but in mutation-negative relatives, an association was established between oral contraceptive use and cancer risk. Although this could suggest a different effect of oral contraceptive use in mutation carriers and non mutation carriers, one should realize that our analysis is complicated by the fact that we analyzed the risk of extracolonic cancer, which in the case of mutation carriers included both breast cancers and endometrial and ovarian cancers and in the case of mutation-negative females included breast cancer but no endometrial and ovarian cancers. The latter is known to show a negative association with oral contraceptive use, in the general population as well as in some studies on ovarian cancer risk in BRCA1 and BRCA2 mutation carriers [8, 20, 22]. Nevertheless, a final advice on whether to use oral contraceptives should be based on overall cancer risk.

As mentioned previously, it has been assumed that mutation negative-relatives of carriers of cancer susceptibility genes might show an increased risk of cancer due to the influence of modifier genes and environmental factors, which makes these families prone to develop cancer and therefore more likely to be screened for mutations involved in cancer susceptibility genes [25, 29]. However, in our cohort, we could only establish a relationship between oral contraceptive use and cancer risk in mutation-negative females.

Risk of extracolonic cancer

In the present analysis, mutation-positive females had a significant higher risk of extracolonic cancer compared to their mutation-negative sisters. This result is in contradiction with our previous report, where a Fisher's exact test was used to analyse the data and no significant difference was found. As discussed in the "Materials and Methods" section, this test was not valid for our study design and therefore the results of the present analysis replace the previous one. We conclude that *h*MLH1 c.C1528T mutation carriers are at an elevated risk for extracolonic cancer with an Odd's ratio of 3.33 (95%CI: 1.11–10.00). Still, this risk seems to be lower than previously reported in other families.

Limitations

Several limitations can be recognized in the present study. Most importantly, the study was constrained by the small size of the cohort and more importantly by the low frequency of extracolonic cancers. It is possible that associations exist, but that our study did not have enough power to detect them. As a consequence, we were not able to analyze the influence of the investigated environmental factors on specific cancer types. Furthermore, the scope of this study was limited to females and extracolonic cancer, with a focus on 'gynaecological factors', instead of the complete LS tumor spectrum in mutation carriers of both sexes. Lastly, as the extracolonic tumour spectrum in our cohort differs from most other families described, they will probably display a different spectrum of environmental factors that influence cancer risk and one can therefore question the possibility of applying our findings to other families.

Although these limitations are serious, we believe that the strength of the present study is the uniqueness of the relatively large 'homogeneous' cohort, as well as its attempt to contribute to a more comprehensive approach of risk management in LS-mutation carriers. Longitudinal follow-up of the c.C1528T cohort might provide us with additional data as the number of mutation carriers and the incidence of extracolonic cancer will rise with time. In addition, further research on the influence of environmental factors in cohorts known to have a high incidence of extracolonic cancer, e.g. in *h*MLH6 mutation carriers, would be worthwhile. Furthermore, investigations of the influence of other environmental factors on CRC and extracolonic cancer risk should be expanded.

Conclusion

In conclusion, no effect of any of the investigated risk factors on extracolonic cancer risk in LS mutation carriers was found in the present study. The effect of environmental factors on disease expression in LS mutation carriers is scarcely investigated. Therefore, this study should be taken as a motivation for further research. Knowledge of the impact of modifiable factors on cancer risk might enable us to provide mutation carriers with evidence-based lifestyle counselling, thereby complementing the prevention strategies available today. Only a broad strategy involving primary and secondary prevention methods will offer mutation carriers the best prospective possible outcomes.

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