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Fertility and apparent genetic anticipation in Lynch syndrome

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Abstract

Background

Genetic anticipation is the phenomenon in which age of onset of an inherited disorder decreases in successive generations. Inconsistent evidence suggests that this occurs in Lynch syndrome. A possible cause for apparent anticipation is fecundity bias, which occurs if the disease adversely affects fertility. The purpose of this study was to determine the effect of age of diagnosis of colorectal cancer (CRC) on lifetime fertility in Lynch syndrome, and whether this can falsely create the appearance of genetic anticipation.

Method

A computer model simulated age of diagnosis of CRC in hypothetical Lynch syndrome carriers and their offspring. The model assumed similar age distribution of CRC across generations (i.e. that there was no true anticipation). Age distribution of CRC diagnosis, and lifetime fertility rates (grouped by age of diagnosis of CRC) were determined from the Australasian Colorectal Cancer Family Registry (ACCFR). Apparent anticipation was calculated by comparing ages of diagnosis of CRC in affected parent- child pairs.

Results

A total of 1088 patients with CRC were identified from the ACCFR. Total lifetime (cohort) fertility was related to age of diagnosis of CRC (correlation coefficient 0.13, P= 0.0001). In the simulation, apparent anticipation was 1.8 ± 0.54 years (P=0.0044). Observed apparent anticipation in the ACCFR cohort was 4.8 ±1.73 years (P = 0.0064). There was no difference in apparent anticipation between the simulate d and observed parent- child pairs (P = 0.89).

Conclusion

The appearance of genetic anticipation in Lynch syndrome can be falsely created due to changes in fertility.

Introduction

Lynch syndrome is the commonest known inherited predisposition to colorectal cancer (CRC). CRC risk is inherited in an autosomal dominant manner, caused by germline mutations in the DNA mismatch repair (MMR) genes *MLH1, MSH2, MSH6* and *PMS2*. The age- and gender specific risk of colorectal cancer is increased 20 to 500-fold [1] and the average age of diagnosis of colorectal cancer decreases from 69 years in the general population [2]to around 45 years[3] in carriers of these mutations.

Genetic anticipation is the phenomenon in which the age of onset of an inherited disorder decreases in successive generations. This has been shown to occur in a number of inherited neurodegenerative disorders, including Huntington disease [4]. There is some evidence, albeit inconsistent, to suggest genetic anticipation for CRC in Lynch syndrome[5] .

The assessment of anticipation for any disease is complicated because it is well known that the false appearance of genetic anticipation may be caused by various forms of ascertainment bias. In cross-sectional studies, individuals from earlier generations are always older than their offspring by definition, and have therefore lived through more of their period at risk. Subjects from later generations who have not yet manifest the disease are excluded from the analysis even though they may have gone on to develop the disease later in their life, thereby lowering the apparent average age of onset in the more recent generation. This is referred to as follow up bias [6]. Similarly,families with predominantly younger diagnoses in later generations are preferentially identified as being at high risk for Lynch syndrome (and are therefore more likely to be included in family cancer registries) compared with families with predominantly older patients in later generations (this occurs because individuals in later generations have not lived long enough to detect cancers that may develop later in their lives [7, 8]).

Another potential cause of apparent anticipation is referred to as fecundity bias. This occurs if the disease adversely affects fertility, so individuals who develop the disease at a younger age are likely to have fewer children than those who are diagnosed at an older age. No previous studies of anticipation in Lynch syndrome (or to our knowledge any inherited cancer) have examined whether fecundity bias can mimic genetic anticipation, and therefore be the explanation for any apparent anticipation.

The purpose of this study was (i) to determine the effect of age of onset of CRC on fertility in Lynch syndrome, (ii) to determine by computer simulation whether observed changes in fertility can falsely create the appearance of genetic anticipation, and to what extent, (iii) to compare the results of that simulation with the observed appearance of genetic anticipation in a large series of families with Lynch syndrome.

Method

The Australasian Colorectal Cancer Family Registry (ACCFR) is a registry of more than 11500 people from 1800 families in Australia and New Zealand [9]. This registry contains CRC families recruited through the Victorian Cancer Registry (960 population-based case-families) and from family cancer clinics throughout Australia and New Zealand (580 clinic-based case-families), as well as families of people without CRC recruited through the Victorian electoral roll (270 control-families). For all family members, personal and family history of cancer, reproductive history, and other lifestyle and personal characteristics were collected by questionnaire. Attempts were made to verify all reports of CRC diagnoses by medical records, pathology reports, death certificates and linkage to national cancer registry and death registry databases.

Individuals who had developed CRC, and who were members of families known to carry MMR gene mutations (Lynch syndrome families) were categorised by age of diagnosis of CRC. Cohort fertility (the mean number of children born to each individual by the end of their reproductive life- defined as over the age of 50 years) was calculated for each age group of CRC diagnosis (for both men and women). We only included subjects born before 1963 in this analysis in order to include only those who had completed their period of potential fertility.

Model design

A computer model was designed to simulate the age of diagnosis of CRC in large numbers of hypothetical MMR gene mutation carrying men and women (first generation) and their offspring (second generation). The model assumed complete follow-up over the lifetime for all individuals, and assumed the age distribution of CRC diagnoses to be the same across generations (i.e. that there was no genetic anticipation). Because follow up was over the entire lifetime of the hypothetical subjects in both generations, and the complete lifetime risk was applied to each subject, follow up (ascertainment) bias will not cause the appearance of genetic anticipation in this model. In this setting any apparent genetic anticipation would be an artefact.

The model generated an equal number of men and women in the first generation, and allocated gender at random (with a 50:50 chance) to the second generation individuals.

Age of diagnosis of first CRC was randomly assigned according to the (gender-specific) observed distribution of age of first CRC diagnosis from the ACCFR cohort. The number of offspring born to each first generation carrier was randomly assigned according to the observed (gender-specific) distribution of lifetime fertility according to the age of diagnosis of CRC as calculated above. Each second generation individual was given a 50% chance of inheriting the MMR gene mutation. For each mutation-carrying child, age of diagnosis of first CRC was allocated in the same way as for their parents. The simulation was run for 1000 first generation subjects. Ages of diagnoses of CRC were compared between the simulated parents and their affected children.

Model validation: observed apparent anticipation in the ACCFR cohort

The appearance of genetic anticipation in the ACCFR cohort was sought by comparing the age of diagnosis of first CRC between parents with their affected children (‘parent- child pairs’). Parent- child pairs were identified if both the parent and child had been diagnosed with CRC. Subjects were included if they were proven mutation carriers, or if their mutation status was unknown but their family was known to carry a MMR gene mutation. This analysis was repeated using only parent- child pairs in whom the children were born more than 80 years ago. This minimizes the chance of incomplete follow up of the children falsely lowering the apparent age of diagnosis of CRC relative to their parents (follow up bias), and is in keeping with the methodology used by Nilbert et al [10]to correct for the birth cohort effect that can falsely create the appearance of anticipation.

Statistical analysis

Mean age of diagnosis of first CRC was compared using the Student’s t- test. A P - value of 0.05 was regarded as statistically significant. Correlation was determined using Spearman’s rank method. Statistical analysis was done using MedCalc for Windows (MedCalc Software, Ostend, Belgium). All results are stated as mean ± standard error of the mean unless otherwise specified.

Ethical approval for the study was granted by the University of Melbourne Ethics Committee.

Results

Cohort fertility in the ACCFR

The ACCFR database contained complete data for 9351 members of 295 families known to carry Lynch syndrome mutations. Of these, 1088 patients (568 men and 520 women) have been diagnosed with CRC. The mean age of diagnosis of CRC was 46.8 ± 14.3 year (46.3 ± 13.3 for the men, and 47.3 ± 15.4 for the women, $P = 0.24$).

A total of 981 (512 male and 469 female) patients with CRC were born before 1963, and the cohort fertility rates were calculated from this group. Cohort fertility grouped by age of diagnosis of CRC is illustrated in figure 1. Total lifetime (cohort) fertility was related to age of diagnosis of CRC in men (correlation coefficient 0.143, $P= 0.0012$), women (correlation coefficient 0.104, $P= 0.04$) and overall (correlation coefficient 0.13, $P= 0.0001$).

Simulation

Using the above parameters, the simulation was run for 1000 first generation mutation carriers. This generated 1169 simulated offspring who were mutation carriers. The mean difference in age of diagnosis of first CRC between simulated parents and their mutation positive offspring (apparent anticipation) was 1.8 ± 0.54 years ($P=0.0044$). Apparent anticipation was similar for male (1.1 ± 0.77) and female (1.9 ± 0.72) simulated parents ($P = 0.44$).

Apparent anticipation in the ACCFR cohort

A total of 461 parent- child pairs with CRC were identified within the ACCFR study cohort. The mean age of diagnosis of first CRC was 51.1 ± 0.63 years in the parent group, and 42.3 ± 0.56 years in their children ($P<0.0001$). When we included only those parent child pairs with a potential follow up of over 80 years (i.e. only subjects born before 1933), 120 parent- child pairs were identified. In this group, the mean age of diagnosis of first CRC was 53.9 ± 0.68 in the parent group, and 49.1 ± 0.67 in their children (apparent anticipation 4.8

±1.73 years, $P = 0.0064$). There was no significant difference in apparent anticipation between the simulated (1.8 years) and observed (4.8 years) parent-child pairs ($P = 0.89$).

Discussion

This simulation demonstrates that the appearance of genetic anticipation in Lynch syndrome can be created due to changes in lifetime fertility in MMR gene mutation carriers with CRC. The apparent anticipation predicted by the model was not significantly different from the observed appearance of anticipation in the AFCCS families with Lynch syndrome which was in keeping with the observed anticipation in large studies of the Danish HNPCC registry of between three and nine years published by Larson et al [11]and Boonstra et al [8].

We observed a marked decrease in lifetime fertility in mutation carriers with early diagnosis of CRC compared with those who developed CRC later in life. For example, women diagnosed with CRC between ages 20 and 24 years gave birth to a mean of 1.2 children in their lifetime compared with women diagnosed with CRC after age 50 years who gave birth to a mean of 2.8 children in their lifetime. The reasons for the reduction in fertility after CRC in these patient groups have not been studied here, but cancer-related mortality and morbidity, the effects of surgery, chemotherapy and radiotherapy, and personal choice can all be expected to play a role. We are unaware of any previous studies that have documented fertility rates after CRC in Lynch syndrome although reduced fertility is well recognized in patients who receive chemotherapy or radiotherapy, and suspected to occur after surgery for CRC [12]. It is this reduction in fertility that contributed to apparent anticipation in our model.

A number of authors have studied genetic anticipation in Lynch syndrome, with differing conclusions as to whether it occurs and to what extent (this literature is summarized in table 1). Tsai et al [13], Westphalen et al[14] and Stupart et al[15] found no evidence of genetic anticipation, but others have reported its occurrence [7, 8, 10, 11, 16-19]. There is variability in the degree of reported anticipation (from three to ten years) even when similar patient registries have been analyzed using different methods.

Paper	**Data set**	**Numbers**	**Average anticipation (years)**
Warthin 1925[16]	"Famliy G"	28 patients with CRC over 4 generations	8
Vasen et al 1994[17]	Foundation for the Detection of Hereditary Tumours (Netherlands)	74 patients with CRC over three generations	8.5
Rodriguez- Bigas 1996[18]	Roswell Park Cancer Institute HNPCC Registry	193 patients with CRC	5.5
Tsai et al 1997[13]	Johns Hopkins Hereditary Colorectal Cancer Registry	67 parent- child pairs with CRC*	0
Voskuil et al 1997[7]	Foundation for the Detection of Hereditary Tumours (Netherlands)	1186 subjects	0
Westphalen et al 2005[14]	University of Basel and Institut Central des Hopitaux Valaisans registrees	55 parent-child pairs with CRC	8
Stella et al 2007[19]	Five Italian families	24 parent-child pairs with CRC	11
Larsen et al 2009[11]	Danish HNPCC Registry	824 subjects	3
Nilbert et al 2009[10]	Danish HNPCC Registry	290 parent-child pairs with CRC	5.5 to 9.8
Boonstra et al 2010 [8]	Danish HNPCC Registry	816 subjects or 290 parent- child pairs with CRC	3 or 8.7
Boonstra et al 2010[8]	University of Michigan Cancer Genetics Clinic	136 parent- child pairs with CRC	9.9
Stupart et al 2013[15]	Single South African family	92 mutation carriers	0

TABLE 1: Previously published studies reporting the presence or absence of genetic anticipation in Lynch syndrome. Boonstra et al [8] described findings from two different patient sets in the same article. Nilbert et al [10] and Boonstra et al [8] found different degrees of apparent anticipation depending on the statistical method used to analyze the data sets.

A potential cause for apparent genetic anticipation is follow- up bias[6]. Thiscan be corrected for by including only subjects who were born sufficiently long ago that they have completed their period at risk[10] (in this case by including only subjects (from both generations) who were born at least 80 years ago). This type of bias cannot fully explain the observed appearance of anticipation in parent-child pairs from the ACCFR as it still existed when we only included mutation carriers with more than 80 years of potential follow up (i.e. born before 1933). This is in keeping with the findings of Nilbert et al [10], whereas Tsai et al [13] found no difference in age of diagnosis of CRC between parents and their children when the birth cohort effect was taken into account.

We included subjects with CRC who were not proven MMR gene mutation carriers (but were from families known to carry MMR gene mutations) as well as those who were confirmed to carry mutations in the MMR genes, in keeping with the methodology of previous authors[10, 13]. This was to allow inclusion of a sufficient number of patients with adequate potential follow up. One would expect the great majority of subjects with CRC born before 1933 to have died before mutation analysis became available. In the ACCFR database, there were 2128 individuals born before 1933, of whom 433 (20.3%) were diagnosed with CRC. Of these 433, only 59 (13.6%) have undergone genetic testing.

Actual genetic anticipation does occur in several neurodegenerative disorders, including Huntington disease[4]and spinocerebellar ataxia [20-22] by the molecular mechanism of generational expansion of trinucleotide repeats during meiosis and gametogenesis [23]. In Li- Fraumeni syndrome, anticipation has been found to be associated with decreasing telomere length over generations[24]. There is no evidence that this occurs in Lynch syndrome, and in a mouse model of Huntington disease MMR gene deficiency has been shown to prevent this from occurring [25]. It has been hypothesised that germline MMR gene defects may lead to an accumulation of small errors in DNA replication prior to loss of heterozygosity which could be passed on over the generations but direct evidence that this occurs is lacking.[23] Bozzao et al [26]have recently described abnormalities in telomere length in carriers of *MSH2* mutations, but not *MLH1* mutation carriers. This is a developing area of research, but a definite molecular mechanism for anticipation in Lynch syndrome (if it occurs) has yet to be found.

In conclusion, we have shown that fecundity bias can falsely create the appearance of genetic anticipation in Lynch syndrome. This highlights the statistical complexity of studying genetic anticipation and the ongoing uncertainty of whether the phenomenon occurs in this disease.

Legend:

FIGURE 1: Cohort (lifetime) fertility rates vs. age of diagnosis of CRC

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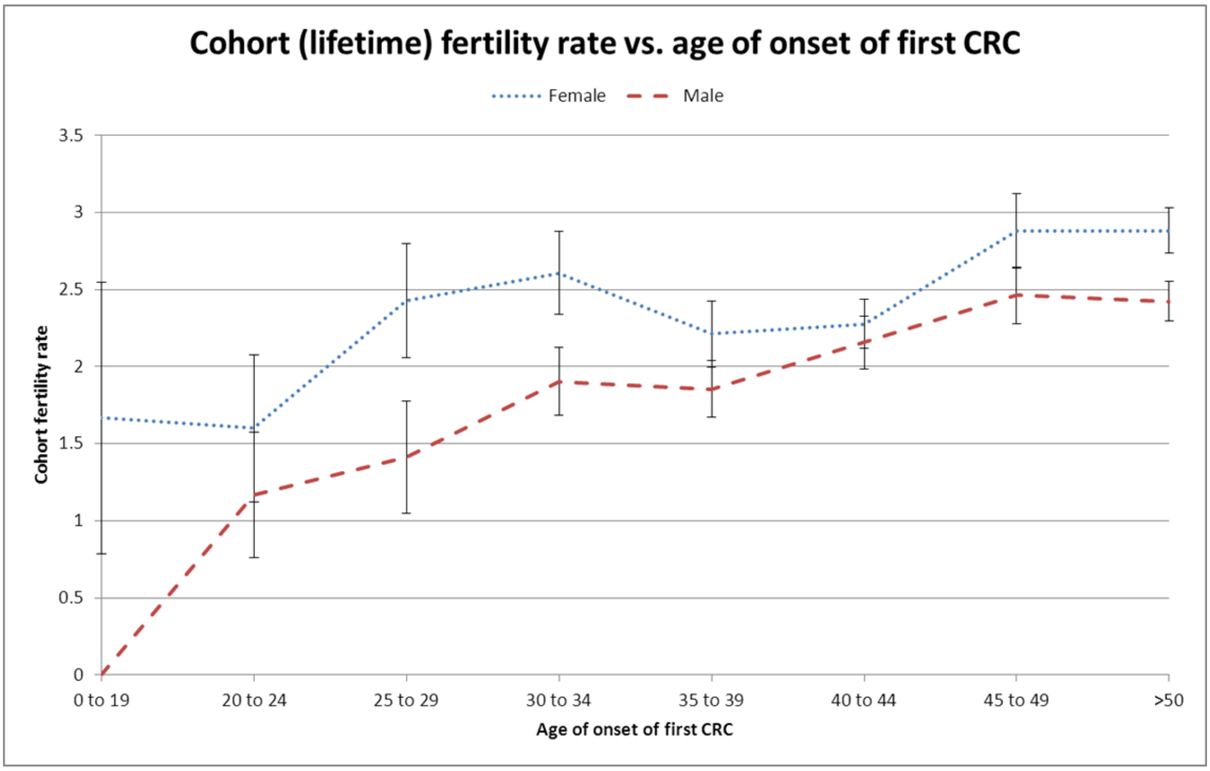
Cohort (lifetime) fertility rate vs. age of onset of first CRC
Female
Male
Cohort fertility rate
3.5
3
2.5
2
1.5
1
0.5
0
0 to 19
20 to 24
25 to 29
30 to 34
35 to 39
40 to 44
45 to 49
>50
Age of onset of first CRC