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Ten Broeke, Sanne W; Rodríguez-Girondo, Mar; Suerink, Manon; Aretz, Stefan; Bernstein, Inge; Capella, Gabriel; Engel, Christoph; Gomez-Garcia, Encarna B; van Hest, Liselotte P; von Knebel Doeberitz, Magnus; Lagerstedt-Robinson, Kristina; Letteboer, Tom G W; Møller, Pål; van Os, Theo A M; Pineda, Marta; Rahner, Nils; Olderode-Berends, Maran J W; von Salomé, Jenny; Schackert, Hans K; Spruijt, Liesbeth; Steinke-Lange, Verena; Wagner, Anja; Tops, Carli M J; Nielsen, Maartje

Published in:
Cancer Epidemiology, Biomarkers & Prevention

DOI (link to publication from Publisher):
10.1158/1055-9965.EPI-18-0576

Publication date:
2019

Document Version
Accepted author manuscript, peer reviewed version

Link to publication from Aalborg University

Citation for published version (APA):
Ten Broeke, S. W., Rodríguez-Girondo, M., Suerink, M., Aretz, S., Bernstein, I., Capella, G., Engel, C., Gomez-Garcia, E. B., van Hest, L. P., von Knebel Doeberitz, M., Lagerstedt-Robinson, K., Letteboer, T. G. W., Møller, P., van Os, T. A. M., Pineda, M., Rahner, N., Olderode-Berends, M. J. W., von Salomé, J., Schackert, H. K., ... Nielsen, M. (2019). The apparent genetic anticipation in PMS2-associated Lynch syndrome families is explained by birth cohort effect. *Cancer Epidemiology, Biomarkers & Prevention*, *28*(6), 1010-1014. https://doi.org/10.1158/1055-9965.EPI-18-0576

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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

The apparent genetic anticipation in PMS2-associated Lynch syndrome families is explained by birth-cohort effect

Running title: Genetic anticipation in PMS2-associated Lynch syndrome families

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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

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Funding: This work was supported by the Dutch Cancer Society [grant number UL-2012-5515], received by Dr. Maartje Nielsen**.**

Conflict of Interest: The authors declare that they have no conflict of interest.

Word count: 2403

Total number of figures: 0

Total number of tables: 3

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ABSTRACT

Background PMS2-associated Lynch syndrome is characterized by a relatively low colorectal cancer (CRC) penetrance compared to other Lynch syndromes. However, age at CRC diagnosis varies widely and a strong genetic anticipation effect has been suggested for *PMS2* families. In this study we examined proposed genetic anticipation in a sample of 152 European *PMS2* families.

Material and methods The 152 families (637 family members) that were eligible for analysis were mainly clinically ascertained via clinical genetics centers. We used weighted Cox-type random effects model, adjusted by birth-cohort and sex, to estimate the generational effect on the age of onset of CRC. Probands and young birth-cohorts were excluded from the analyses. Weights represented mutation probabilities based on kinship coefficients, thus avoiding testing bias.

Results Family data across three generations, including 123 CRCs, were analyzed. When compared to the first generation, the crude Hazard Ratio (HR) for anticipation was 2.242 (95%CI: 1.162-4.328) for the second and 2.644 (95%CI: 1.082-6.464) for the third generation. However, after correction for birth-cohort and sex the effect vanished (HR=1.302 (95%CI: 0.648-2.619) and HR=1.074 (95%CI: 0.406-2.842) for second and third generations, respectively).

Conclusions Our study did not confirm previous reports of genetic anticipation in *PMS2*-associated Lynch syndrome. Birth-cohort effect seems the most likely explanation for observed younger CRC diagnosis in subsequent generations, particularly since there is currently no commonly accepted biological mechanism that could explain genetic anticipation in Lynch syndrome.

Impact This new model for studying genetic anticipation provides a standard for rigorous analysis of families with dominantly inherited cancer predisposition.

KEY WORDS: modifier, penetrance, colon cancer, HNPCC, phenotype

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INTRODUCTION

Lynch syndrome is the most common cause of hereditary colorectal cancer, accounting for 3-5% of all colorectal cancers diagnosed annually.[1] The underlying cause is a heterozygous pathogenic germline variant in one of the mismatch repair genes: *MLH1*, *MSH2* (*EPCAM*), *MSH6* or *PMS2*. The latter gene is associated with a lower estimated penetrance and thus a markedly lower incidence of cancer. However, PMS2 families show phenotypic variability, with very wide differences in age at colorectal cancer diagnosis. While the mean age of onset for colorectal cancer for PMS2-associated Lynch syndrome is around 60, some PMS2 carriers develop colorectal cancer as early as 23.[2-4] Several external and internal modifiers have been suggested as possible explanations, one of which, genetic anticipation, has been the subject of much debate.[5-9] The phenomenon of genetic anticipation is clearly defined in genetic disorders involving trinucleotide repeats such as Huntington's disease, where expansion of the repeat in subsequent generations is a clear precursor of disease.[10] However, a mechanism of this type has not been described in Lynch syndrome, which in fact requires a second somatic hit for mismatch repair (MMR) deficiency to occur. A single germline mutation in one of the MMR genes does not confer haploinsufficiency.[11]

Nevertheless, genetic anticipation in Lynch syndrome and other dominantly inherited cancer predisposition syndromes has been reported by several groups. If a genetic anticipation effect could indeed be confirmed it would be of clinical utility in the development of individually-tailored surveillance schemes. The only report of genetic anticipation in PMS2-associated Lynch syndrome families found a very strong effect (anticipation of 7.3 years per subsequent generation).[12] However, sample size in that study was small, including only 12 PMS2 families. In the same study, carriers of pathogenic germline variants in other MMR genes showed only small or absent anticipation effects.[12] By investigating a much larger cohort of 152 families, our aim was to reassess the possibility of genetic anticipation in PMS2-associated Lynch syndrome.

MATERIALS AND METHODS

Description of the cohort

Pedigree data on European families carrying a segregating pathogenic PMS2 variant were originally collected from clinical genetic departments between 2009 and 2012, as previously described.[4] Further families were collected between 2012 and 2017 and an extensive description is available elsewhere (ten Broeke et al, 2018,

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in-press at Journal of Clinical Oncology). The PMS2 families included originated from the Netherlands, Norway, Germany, Sweden, Denmark and Spain. Data collection was approved by the local ethical review board (Leiden University Medical Center Ethics Review Board, protocol ID: P01.019). This dataset consisted of clinically ascertained families where variant analysis was initiated due to (histological) pre-screening by immunohistochemistry and/or microsatellite instability, usually because a family met Bethesda criteria.[13] Data collection from patient records included demographic data, family pedigrees, age and location of cancer diagnosis, polypectomy, and hysterectomy if applicable. When available, clinical and pathological diagnoses were confirmed using patient records.

Statistical analysis

The outcome of interest was age at first diagnosis of colorectal cancer. The follow-up time was defined as the time elapsed from birth till the first colorectal cancer diagnosis or censoring. Censoring occurred on the basis of last known other cancer diagnosis, death or administrative censoring at age of last contact with the family, whichever occurred last. Family members with bi-allelic PMS2 mutations were excluded from the analysis given the severe and markedly different phenotype of these constitutional mismatch repair deficiency (CMMRD) patients. Genetic anticipation was estimated as the effect of generation on a person's hazard for cancer diagnosis, using a shared gamma frailty proportional hazard model:

$$\lambda(t_{ij}) = u_i \lambda_0(t_{ij}) \exp(\boldsymbol{\beta Z}_{ij} + \boldsymbol{\gamma X}_{ij}),$$

where t_{ij} is the age at first diagnosis of colorectal cancer or the age at censoring for member *j* in family *i*, $\lambda_0(t_{ij})$ refers to the baseline hazard, which is left completely unspecified (Cox-type model), $\boldsymbol{\beta} = (\beta_1, \beta_2)$ contains the main effects of interest, the regression coefficient of second and third generation $\boldsymbol{Z} = (Z_1, Z_2)$, taking the first oldest generation of each family as reference and $u > 0$ refers to an unobserved random effect (frailty) shared by the members of the same family. This unobserved heterogeneity shared within families was assumed to follow a gamma distribution (normal frailty was also checked as a sensitivity analysis). $\boldsymbol{\gamma}$ contains the effect of person-specific covariates $\boldsymbol{X}$ included in a second adjusted analysis, namely sex and year of birth.

Since not all family members were tested for *PMS2* variants, mutation probabilities based on kinship coefficients were used as analytical weights to avoid possible testing bias and increase efficiency. Specifically, the weight for individual *j*, $w_j = P(mutation|family\ history\ of\ mutation)$ is given by the kinship

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coefficient between individual *j* and the closest family member with observed mutation. Mutation probabilities are included as case weights in the corresponding penalized score function provided in the R package *survival*.[14] Remaining ascertainment bias was controlled by excluding the probands and focusing on individuals born before 1950, so that all included individuals were at risk for at least 65 years, hence avoiding potential bias due to right truncation. Statistical significance was established at 5%.

RESULTS

A description of the cohort is given in tables 1 and 2. The analysis included 637 family members with 123 colorectal cancers (table 1), divided over 3 generations (table 2). After weighting, the estimated number of mutation carriers in the sample is 360. Results of the Cox-type random effects model are given in table 3, which shows increased hazard ratios (HRs) in the crude analysis (HR=2.24, 95% CI=1.16-4.33 for the second generation and HR=2.64, 95% CI=1.08-6.46 for the third generation, respectively). After correction for gender and birth-cohort, HR size decreased (half of the crude effect) and was no longer statistically significant (as the corresponding confidence intervals included 1). The adjusted analysis showed a strong effect of year of birth (HR=1.05, 95% CI = 1.02-1.07), equaling a roughly 5% increase of risk for every year towards the present time. These results suggest that the estimated anticipation effect in the crude analysis is strongly confounded by birth-cohort and that the apparent effect of generation is mainly explained by secular trends in colorectal cancer diagnosis. The use of normal random effects instead of gamma provided very similar results in terms of genetic anticipation, sex and birth-cohort effects (results available in supplementary table 1).

DISCUSSION

The occurrence of genetic anticipation in Lynch syndrome has been a subject of considerable debate and gene-specific effects have been offered as an explanation. After correction for birth-cohort, our analysis found no evidence of anticipation in a very large cohort of PMS2-associated Lynch syndrome families. A rise in colorectal cancer incidence as well as lower age at diagnosis in recent decades in the general population has been previously observed.[15-17] Reasons for this might include better detection with more sensitive screening methods, lifestyle factors, population-based screening protocols and increased life-expectancy. These factors could also play a role in Lynch syndrome patients. Other factors that could cause a false genetic anticipation

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signal that are specific to Lynch syndrome, and other dominantly inherited cancer predisposition syndromes in general, involve the genetic diagnostic process. For example, after identification of the proband, pre-symptomatic family members are tested and subsequently screened if they carry the *PMS2* variant. This might lower age at diagnoses of indolent tumors which might not have presented itself otherwise. An alternative explanation for false genetic anticipation effect may be that colorectal cancer diagnosis in older generations may have been underreported.

Analysis of dominantly inherited cancer predisposition is potentially influenced by several forms of bias. First, clinically ascertained families are accompanied by a selection bias, as they were selected due to their compliance with clinical selection criteria and are therefore often severely affected, i.e. many family members with (colorectal) cancer or an unusually low age at diagnosis. A problem arises when the phenotype is not caused by the pathogenic *PMS2* variant alone but is affected by other modifying factors. This is especially problematic for PMS2, as selection based on, for example, the Bethesda guidelines is influenced by criteria for classic Lynch families involving mainly pathogenic *MLH1* or *MSH2* variants. In the case of *PMS2* variants, it is well documented that variants are at most only moderately penetrant[2, 4], suggesting that *PMS2* families selected on the basis of these criteria alone will include many relatively severely affected members. However, due to universal screening for mismatch repair deficiency in all colorectal cancers below age 70 in most Western countries, a rise in unselected *PMS2* carriers is expected.[18]

A second form of bias that should be considered is testing bias due to the fact that people affected with (colorectal) cancer (at a young age) are more likely to be tested for the presence of a *PMS2* variant. Probands (i.e. the first person in the family with a confirmed pathogenic germline *PMS2* variant) are the most notable example of this, and all probands were therefore excluded from our analysis. Moreover, we also used analytical weights to model mutation probabilities. For example, first-degree relatives of a confirmed carrier that were not tested were given a weight of 0.5, whereas second-degree relatives had a weight of 0.25. This approach also helped improve the power of the analysis.

Although there is no clear biological rationale for genetic anticipation in Lynch syndrome, alternative explanations besides birth-cohort have been proposed in other studies. It is generally accepted that families with Li-Fraumeni syndrome (which strongly predisposes to several forms of cancer) exhibit anticipation that cannot be explained by a birth-cohort effect.[19, 20] A recent whole genome sequencing study of germline DNA in

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13 Li-Fraumeni syndrome cases did not find increased DNA copy-number variations, suggesting that CNVs do not mediate the genetic anticipation effect. The authors proposed an alternative model explaining apparent anticipation in which variants from the non-carrier parent influence tumorigenesis in the offspring of TP53 mutation carriers with late onset of cancer.[21] In other words, parents with relatively late onset might have offspring that are more prone to tumorigenesis due to inheritance of specific risk increasing variants from the non-carrier parent. Similar mechanisms may also influence cancer age of onset and thus explain variability within families and birth-cohorts in Lynch syndrome. Another suggested biological mechanism involves telomeres. Retrospective studies have identified shorter telomeres in colorectal cancer cases vs. controls, arguing that shorter telomeres cause chromosomal instability and might therefore lead to cancer. Indeed, shortening of telomeres was also observed in peripheral blood in Lynch Syndrome patients affected with colorectal cancer, compared to non-affected mutation carriers.[22] This finding has not been replicated in prospective studies, suggesting that the shortening of telomeres might be the result of the cancer process rather than a causative factor.[23]

Ours is not the first study to report bias in anticipation analysis due to birth-cohort effects. Similar results have been found in other genetic syndromes, including a study by Guindalini et al. in BRCA1/2 families.[24] This study corrected for various types of bias by excluding probands, including mutation probabilities and correcting for birth-cohort. Our analysis followed similar principles and incorporated additional flexibility in the specification of the regression model. Our model is semi-parametric, since the baseline hazard is left completely unspecified and is therefore more flexible than the model used by Guindalini et al., which was based on a parametric specification of the underlying time-to-event data distribution.[25] Moreover, we have allowed for a more flexible, non-linear effect of generation, considering two possibly different effects for second and third generations with respect to the first, oldest generation. Previous reports have relied on a linear and perhaps too stringent specification of the anticipation effect. We also used gamma random effects in our main analyses and checked the impact of random effect specification by also considering normal random effects. The results regarding anticipation and birth-cohort effect remained the same. Normal random effect modeling of hazard was previously used by von Salome et al. in a study in which the authors reported strong genetic anticipation in twelve PMS2 families.[12] However, cohort effects were not considered and a linear specification was assumed for the generation effect. Daugherty et al. also used a Cox-type hazard

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regression method to study anticipation in lymphoproliferative tumors but adopted a less flexible approach since random effects were not considered and hence family-specific effects could not be captured.[26] Nevertheless, these authors also identified a confounding effect of secular trends on apparent anticipation effects of generation.

Regression strategies have previously been shown to be preferable over hypothesis testing based on parent-child pairs.[9] Since our regression strategy is flexible, it is possible to reasonably reflect the underlying structure of the data while still getting interpretable results and preserving sufficient power. Boonstra et al. have reported genetic anticipation in Lynch syndrome based on an alternative specification that allowed for family-specific anticipation effects (random slopes).[27] Such specification is flexible since it allows for a specific effect of generation in each family, although the effect is linear within families. We have introduced flexibility in a different manner, by allowing for a non-linear fixed anticipation effect, which is less dependent in the chosen parametric family on random effects. Moreover, Boonstra et al. did not directly estimate cohort effects based on the sample, but inferred them from external cancer incidence registries (not specific for Lynch syndrome) on the basis of a piecewise (5-year knots) linear hazard assumption.[27] Misspecification in this step may have introduced bias in the estimated anticipation effect. Despite our efforts to account for possible bias in our analysis strategy, the retrospective nature of our data is still a limitation of our study. Similarly, in an effort to avoid ascertainment bias we excluded some data, leading to a reduction in power. Models which can accommodate right truncated data should be developed and used in this field. A last limitation is that the weights that were used to estimate the probability of carrying the familial *PMS2* mutation only took into account degree of kinship, but not the presence of a cancer phenotype, e.g. colorectal cancer. Including this factor in the weigh calculation is complicated given the complex pedigree structure. Moreover, recent work by our own group suggests that the lifetime risk for colorectal cancer is only 2-3 times increased compared to the general population (ten Broeke et al, 2018, in-press at Journal of Clinical Oncology), which may cause misspecification of PMS2-associated colorectal cancer as this cancer also occurs frequently in the general population.

In conclusion, after correction for birth-cohort, our study did not confirm previous findings of genetic anticipation in PMS2-associated Lynch syndrome patients. Therefore, anticipation cannot be used in individual

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risk estimation. Given the large phenotypic variability in Lynch syndrome patients, future studies should focus on other potential modifiers.

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TABLES

Table 1: Cohort description

Number of families	152
Family members included	637
Mutation status	
100%*	176
[50%,100%)	282
[25%,50%)	158
[12.5%,25%)	21
Colorectal cancer	
Number	123
Mean Age (s.d.)	69.58 (12.94)
Median age (IQR)	71 (62-77)

s.d.: Standard deviation; IQR: Interquartile range.

* Confirmed and obligate carriers.

Note: Probands were excluded from the analysis.

Table 2: Number of family members for each generation and median year of birth

Generation	Number	Median (IQR)
1	153	1912 (1902-1924)
2	399	1927 (1918-1938)
3	85	1943 (1937-1950)

IQR: Interquartile range

Table 3: Results of Cox model

	Crude analysis		Adjusted analysis*	
	HR	**95% CI**	**HR**	**95% CI**
Generation 1	*reference*		*reference*	
Generation 2	2.24	1.16 to 4.33	1.30	0.65 to 2.62
Generation 3	2.64	1.08 to 6.46	1.07	0.41 to 2.84

*Adjusted for: gender and year of birth

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Author Manuscript Published OnlineFirst on March 1, 2019; DOI: 10.1158/1055-9965.EPI-18-0576
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

The apparent genetic anticipation in PMS2-associated Lynch syndrome families is explained by birth cohort effect

Sanne W. ten Broeke, Mar Rodríguez-Girondo, Manon Suerink, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst March 1, 2019.

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