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Genetic anticipation in *BRCA1/2* families after controlling for ascertainment bias and cohort effect

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Abstract

Background—Genetic anticipation, the earlier onset of disease in successive generations, has been reported in hereditary breast and ovarian cancer syndrome (HBOC), but little is known about its underlying mechanisms. Ascertainment bias is a reason that has been suggested in previous studies. Likewise, cohort effect, which may be due to environmental factors, can be misinterpreted as genetic anticipation.

Methods—We conducted a review of pedigrees in 176 kindreds segregating deleterious mutations in *BRCA1/2* genes who had at least two consecutive generations of the same cancer (breast or ovarian). Using mutation probabilities as analytical weights in weighted random-effect models, we calculated generational differences in age of onset of breast/ovarian cancer. Then we further controlled for ascertainment bias by excluding probands and adjusted for birth cohort effect in the anticipation models.

Results—The mean age of breast cancer for the probands' generation was 41.9 years, which was 6.8 years and 9.8 years earlier than the parents' and grandparents' generations, respectively. The anticipation effect for breast cancer remained significant after excluding probands. There was a birth cohort effect: patients born in 1930s and 1940s had breast cancer 5.0 and 7.6 years earlier than patients born before 1920. The difference in breast cancer age of onset across generations was no longer significant after adjusting for birth cohort effect.

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Conclusions—The observed anticipation effect was mainly driven by a decrease in age of onset across birth cohorts, underscoring the need for risk reducing interventions that target changing environmental/lifestyle factors in *BRCA1/2* carriers.

Keywords

ascertainment bias; *BRCA1/2*; cohort effect; genetic anticipation; hereditary breast and ovarian cancer syndrome

INTRODUCTION

Genetic anticipation is characterized by a reduction in the age of disease onset or an increase in severity of clinical features of a genetic disorder in successive generations. This phenomenon has been studied in a range of diseases, including neurodegenerative disorders such as fragile X syndrome¹, Huntington's disease², and myotonic dystrophy³, and autoimmune diseases such as rheumatoid arthritis⁴, type II diabetes⁵, and Grave's disease⁶. Genetic anticipation has previously been described in several cancer predisposition syndromes, such as retinoblastoma⁷, Lynch Syndrome^{8,9}, Li-Fraumeni Syndrome^{10,12}, and hereditary breast and ovarian cancer syndrome (HBOC)^{13,22}.

Globally, breast cancer is the most common form of cancer in women. Numerous genetic and non-genetic factors affect the likelihood of developing breast cancer, but no other predictor is as powerful as an inherited mutation in the tumor suppressor genes *BRCA1/2*. More than two decades after these genes were identified, we now know that individuals carrying mutations in *BRCA1* and *BRCA2* have an average cumulative lifetime risk of 44-78% and 31-56% for breast cancer and 18-54% and 2.4-19% for ovarian cancer, respectively, making these mutations the strongest breast and ovarian cancer predictors.^{23,32} It is estimated that approximately 5-10% of breast cancer cases are thought to be hereditary²⁷. While other breast cancer susceptibility genes have been identified, mutations in *BRCA1/2* account for nearly 80% of inherited breast cancer cases in HBOC³³. The variable penetrance of inherited mutations in these genes makes it challenging to estimate the likelihood, age, and site of disease onset in any individual, and therefore it is important to study how to time the initiation of screening and interventions in high-risk women. Several studies examined the anticipation effect in HBOC, reporting a 4 to 18 year younger age of breast cancer onset in successive generations^{13,22}. However, most of these studies were based on very small or genetically uncharacterized cohorts. Although there is evidence for the existences of anticipation in HBOC, this phenomenon is not firmly accepted by the scientific community for three reasons: ascertainment bias, cohort effect, and unclear molecular mechanism³⁴.

One of the major concerns when assessing genetic anticipation in HBOC is ascertainment bias. The nature of the ascertainment introduces a possible younger onset age bias in that mutation carriers suffering from early onset breast cancer are more likely to fulfill the criteria for genetic testing than mutation carriers with late-onset breast cancer. Within the same family, members with early onset cancer are more likely to be the first to present in the high risk clinics for genetic counseling, i.e. becoming the proband who brings the family

under the study. As a consequence, ascertainment of mutation carriers in high-risk clinics may lead to an overrepresentation of earlier age of disease onset in younger generation than the older generation. Various statistical approaches can be employed to reduce the impact of this ascertainment bias, such as evaluating not only parent-child pairs but also all the affected family members as well as excluding probands from the calculation. In order to address this issue, Larsen *et al*³⁵ proposed a random effects model which includes extended pedigree data and unaffected family members. Still, new methods are needed to account for family members who are not tested.

With respect to the cohort effect, penetrance of the disease also depends on nongenetic influences, which can be assessed through the year of birth of mutation carriers. In 2003, King *et al.* demonstrated that the cumulative risk of breast cancer was significantly higher in *BRCA1/2* carriers born after 1940 compared to those born before 1940²⁹. This birth cohort effect may be due to improvements in cancer programs in high-risk families over time as well as secular changes in environmental and lifestyle risk factors for breast or ovarian cancers.

One molecular basis of genetic anticipation was first discovered in neurodegenerative disorders. Brook *et al.* observed expanded trinucleotide repeat sequences in myotonic dystrophy with expansion of repeats clearly correlated with an earlier age of onset in succeeding generations³⁶. Trinucleotide repeat expansions is now a widely accepted explanation of this phenomenon in some Mendelian diseases. In cancer, the molecular mechanisms behind anticipation likely differ since trinucleotide expansions have never been observed. Recently, telomere shortening has been suggested as an alternative mechanistic explanation. Martinez-Delgado *et al.* reported a positive correlation between decrease of the telomere length and anticipation in 19 mother-daughter pairs from breast cancer families²¹. This finding is valuable, but the small number of subjects in the study and the use of paired t-tests without correcting for potential bias calls for larger studies for replication. In addition, the hypothetical causal relationship between telomere shortening and genetic anticipation was not found in a study evaluating 244 mismatch repair gene mutation carriers, and it is still under debate in Lynch syndrome³⁷.

Given these controversies, we conducted a relatively large study of families segregating deleterious *BRCA1/2* mutations to evaluate the difference of breast/ovarian cancer onset in successive generations using a novel sophisticated statistical approach to adjust for ascertainment bias and cohort effect.

METHODS

Study population

We reviewed pedigrees of consecutive families ascertained in the Cancer Risk Clinic at The University of Chicago between 1992 and 2013 under IRB approved protocols. To be eligible for the study, families had to have a confirmed *BRCA1* or *BRCA2* deleterious mutation and at least two family members diagnosed with the same cancer (breast or ovarian) in consecutive generations. Of note, the probands did not necessarily have to have been diagnosed with cancer. Clinical and epidemiological data on the affected and unaffected

members in a family such as family members' age, ages at death, types of cancer, age at cancer diagnosis, and genetic test results were collected.

Statistical analysis

The study outcome variable was age at breast or ovarian cancer diagnosis. The primary variable of interest was generation. Other covariates included year of birth and type of mutation. To include family members without genetic testing in the model, we calculated the probability of having *BRCA1/2* mutation using the BRCAPRO program³⁸. The mutation probabilities, or BRCAPRO scores, were used as analytical weights for all family members with breast or ovarian cancer with unknown *BRCA* status, and patients with known positive mutation status were given a weight of one. Then we applied these analytical weights to the parametric random-effect model for anticipation proposed by Larsen et al³⁵. The basic model is presented below:

$$T_{ij} = \alpha_i + m_{ij}\gamma + \mathbf{x}_{ij}\beta + \varepsilon_{ij}$$

Where, T_{ij} denoted age at diagnosis of individual j in the i th family; m_{ij} denoted the generation; $\mathbf{x}_{ij} = (x_{ij1}, x_{ij2}, \dots, x_{ijk})$ indicated K covariates. γ was the effect of generation, the parameter of interest in the study; α_i was the family-specific intercept, and ε_{ij} represented the residuals; both were random terms, assuming normal distribution. The use of mutation probabilities as analytical weights not only improves the statistical power by including all cancer patients in the families, but also reduces potential ascertainment bias. Furthermore, we excluded probands from the model to control for ascertainment bias, as probands are more likely to be self-selected for genetic counseling because of earlier age at diagnosis. To determine whether the genetic anticipation effect is independent of birth cohort effect, we adjusted for year of birth as a covariate in matrix K . We analyzed the data separately for breast cancer and ovarian cancer and further stratified according to genes with mutation (*BRCA1* vs. *BRCA2*).

RESULTS

Study population

Of the 532 families who have been tested for *BRCA1/2* in our database, 300 families had at least one deleterious *BRCA1/2* mutation segregated. Of these, 176 (34.9%) met the inclusion criteria that two or more family members have the same cancer (breast or ovarian) in consecutive generations. Characteristics of the probands and their family members diagnosed with cancer are described in Table 1. There are 113 families with *BRCA1* mutations and 63 families with *BRCA2* mutations. In 155 families, at least two family members had breast cancers, and a total of 579 family members were included in the anticipation analysis of breast cancer. Using the BRCAPRO program, we estimated mutation probabilities for patients without *BRCA1/2* testing, and predicted the total number of mutation carriers among breast cancer cases was 469. In 36 families, at least two family members developed ovarian cancer and a total of 81 family members are included in the anticipation analysis of ovarian cancer. The predicted total number of mutation carriers

among ovarian cancer patients was 78. Note that 15 families were included in breast cancer and ovarian cancer families analyses.

Anticipation effect with probands included

In the random-effect model accounting for mutation probabilities and family structure, we found there was a significant anticipation effect for breast cancer among *BRCA1/2* mutation carriers (Table 2). Relative to the probands' generation, parents' generation had breast cancer 6.8 years later, and grandparents' generation had breast cancer 9.8 years later, while the children's generation had breast cancer 7.2 years earlier. Subgroup analysis found that the anticipation effect for breast cancer was significant among families with either *BRCA1* or *BRCA2* mutations (Supplementary Table 1).

When evaluating differences in ovarian cancer age of onset between generations, we found significant anticipation effect for ovarian cancer although the sample size is limited (Table 2): The grandparents' generation and parents' generation were estimated to have ovarian cancer 8.4 years or 4.8 years, respectively, older than the probands' generation, while the children's generation had ovarian cancer 10.5 years earlier than the probands' generation.

Anticipation effect after excluding probands

After correcting for ascertainment bias by excluding probands (families that did not have at least 2 generations after excluding probands were also excluded from the analysis), the mean age of breast cancer onset in the probands' generation was 41 years. We found that breast cancer age of onset in the probands' generation was 7.0 years and 10.5 years earlier than in the parents' and grandparents' generations, respectively. These differences remained statistically significant, suggesting that ascertainment bias cannot explain the observed anticipation effect (Table 3). Subgroup analysis showed that the anticipation effect for breast cancer existed in both *BRCA1* (mean age of onset – probands' generation: 40 years; parents' generation: 45.1 years; grandparents' generation: 50.7 years) and *BRCA2* (mean age of onset – probands' generation: 42.8 years; parents' generation: 53 years; grandparents' generation: 52.7 years) mutation families (Supplementary Table 2).

After excluding probands, the anticipation effect for ovarian cancer remained statistically significant, with grandparents' generation having age of onset 8.6 years older than the probands' generation (Table 3).

Jointly modeling anticipation effect and birth cohort effect

Mutation carriers born after 1950 (mean 1960, range 1950-1981) reached age ranging from 32 to 63 by year 2013, and thus some of them may develop breast or ovarian cancers later. Therefore we excluded birth cohorts after 1950 from the analysis in order to avoid truncation effect. In the univariate random effect model, the anticipation effect was still significant but the effect size was smaller than that in the model of all breast cancer patients (Table 4). The 1930-1949 birth cohorts were more likely to have early-onset breast cancer than the 1855-1919 birth cohort. After adjusting for birth cohort effect in the multivariable random effect model, the anticipation effect (i.e. generational effect) was no longer significant, while the birth cohort effect remained statistically significant. Similarly, we found a birth cohort

effect for ovarian cancer and the anticipation effect was not significant after adjusting for birth cohort effect, but the sample size for ovarian cancer analysis was limited (Table 4).

DISCUSSION

In the current analysis, we evaluated multigenerational families with deleterious *BRCA1/2* mutations, adjusting for ascertainment bias and cohort effect concomitantly and using individual probabilities of having *BRCA1/2* mutations as analytical weights to address and report anticipation phenomenon in HBOC. In agreement with previous reports, we detected statistically significant anticipation effect in breast and ovarian cancers in *BRCA1/2* families in our primary analysis (Table 5) and adjusted analyses for ascertainment bias. Nevertheless, as expected, potential biases had a profound influence on these results. By applying statistical approaches, we showed that the significant decrease in age of breast cancer diagnosis over time is due to cohort effect, eliminating the signal of anticipation in breast and ovarian cancer families. This finding suggests that environmental/lifestyle factors rather than genetic changes account for the anticipation effect.

Our study found that mutation carriers born after 1930 had younger onset of breast and ovarian cancers than carriers born before 1930, which is in line with previous studies among *BRCA1/2* carriers.^{29,39} In general, there is evidence of an increase in breast cancer incidence rates in the recent birth cohort, especially in traditionally low risk regions such as Africa, Asia, and South America where rapid urbanization and modernization are underway in the past several decades.^{40,41} This trend likely reflects increasing prevalence of nongenetic risk factors for breast cancer associated with lifestyle changes as well as increased life expectancy and increased detection of asymptomatic disease after mammography screening became routine.^{40,41} The most commonly implicated nongenetic risk factors for the overall increase in breast cancer risk among women born more recently are changes in female reproductive patterns (lower fertility and delayed childbearing), earlier age of menarche, increasing use of oral contraceptives and menopausal hormone therapy, increasing consumption of calorie-dense food and alcohol, physical inactivity, and obesity.⁴² These factors may have had a similar overall effect between *BRCA* gene mutation carriers and women of the general population.^{29,32,39,43} A recent meta-analysis on modifiers in *BRCA1/2* mutation carriers showed that age at first live birth, breastfeeding, tubal ligation, oral contraceptives, and smoking are possible risk modifiers.⁴¹ Further investigation of risk modifiers in mutation carriers based on large consortium will help to fine tune cancer risk estimation.

Several reports have claimed statistical evidence that earlier appearance of breast cancer in successive generations occurs in HBOC due to generational effects. We approached this claim with skepticism. To date, the only universally accepted molecular mechanism to explain anticipation phenomenon in a variety of neurodegenerative diseases – trinucleotide repeat sequences expansion – has never been described in HBOC. In addition, there are no definitive conclusions about an alternative mechanistic explanation such as telomere shortening. Although the positive correlation between the phenomenon of anticipation and telomere shortening has been described, this finding is inconsistent among cancer predisposition syndromes.^{21,37,44}

Furthermore, haplotype analysis suggests that the origin of some *BRCA1/2* founder mutations probably occurred at least 50 or more generations ago (>1000 years ago)^{45,46}. Considering this estimation, such a large decrease (4 to 18 years) in age of breast cancer onset in successive generations as previously reported ought to lead to juvenile onset cases within a few generations. Despite the fact that juvenile onset cases have been observed in later generations in repeat expansion disorders, no such cases have been observed in *BRCA1/2* families.

We acknowledge that the current sample size is still modest and even though a series of statistical approaches have been undertaken to control for several biases, our results must be viewed cautiously given unavoidable biases attending retrospective analyses such as mutational bias and information bias. In order to minimize mutational bias, BRCAPRO scores were used as analytical weights to estimate individual risks of having *BRCA1/2* mutations because individuals in earlier generations often did not have genetic testing. To improve the quality of information, the initial counseling session at our institution is preceded by a phone call interview with a genetic counselor. During this conversation, probands are encouraged to gather important information of family members regarding their cancer history in advance as well as to provide pathology reports of affected family members when available. Detailed pedigrees are drawn at the time of the initial visit in a systematic manner using Progeny software (<http://www.progenygenetics.com>) and are updated at each visit. Lastly, the finding that early-onset of breast cancer in the children's generation should be interpreted with caution since they were born relatively recently (mean year of birth was 1961) and some of them may not have chance to develop cancer yet.

In conclusion, the prediction of age of cancer onset in those carrying mutations causal of HBOC has clinical implications for the age at which surveillance should be initiated and is important for clinical trial design and understanding of fundamental disease mechanisms. Although it has been suggested that genetic anticipation occurs in HBOC, there is no confirmed molecular mechanism to support it, and cohort effect may confound this interpretation. In our study that corrected for these biases, the statistical trend toward earlier age of onset in successive generations was eliminated, indicating that the cohort effect drove the apparent observation of genetic anticipation. Nonetheless, the observed cohort effect has implications for cancer prevention. Interventions to reduce risk by modulating lifestyle/environmental risk factors such as breastfeeding may be warranted after family based counseling. Our findings also indicate that it is worthwhile to review and apply rigorous statistical approaches in studies addressing anticipation in order to reduce the influence of potential biases in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of Study Samples

	Breast cancer family*	Ovarian cancer family[†]	Total[‡]
Number of families	155	36	176 [*]
Number of families with <i>BRCA1</i> mutation	94	29	113
Number of families with <i>BRCA2</i> mutation	61	7	63
Number of family members with breast cancer	579	74	587
Predicted number of mutation carriers in breast cancer patients	469.1	60.5	477.1
Number of family members with ovarian cancer	59	81	105
Predicted number of mutation carriers in ovarian cancer patients	56.5	77.7	111.3
Probands with breast cancer	96	12	100
Probands with ovarian cancer	10	8	14
Probands with breast and ovarian cancer	7	3	8

* at least two family members diagnosed with breast cancer in consecutive generations;

[†] at least two family members diagnosed with ovarian cancer in consecutive generations.

[‡] 15 families were included as both breast and ovarian cancer families, so the numbers cannot be added up.

Table 2Anticipation effect in families with *BRCA1/2* mutations

Generation	Breast Cancer (N=579)				Ovarian Cancer (N=81)			
	N	Mean AoO [†]	AoO (95% CI)	P-value	N	Mean AoO [†]	AoO (95% CI)	P-value
Grandparents' gen	81	51.8	9.8 (6.3, 13.4)	<0.001	14	57.1	8.4 (1.8, 15.0)	0.013
Parents' gen	225	48.7	6.8 (4.6, 9.1)	<0.001	36	53.5	4.8 (0.1, 9.5)	0.048
Probands' gen	242	41.9	0 (ref)		27	48.8	0 (ref)	
Children's gen	31	34.7	-7.2 (-11.8, -2.7)	0.002	4	38.3	-10.5 (-20.4, -0.6)	0.038

[†] mutation probability-weighted age of onset in years

Abbreviations: : AoO, difference in years of age of onset; CI, confidence intervals; gen, generation; ref, reference.

Table 3Anticipation effect in families with *BRCA1/2* mutations after controlling for ascertainment bias

	Breast Cancer, probands excluded (N=447)				Ovarian Cancer, probands excluded (N=67)			
Generation	N	Mean AoO [†]	AoO (95% CI)	P-value	N	Mean AoO [†]	AoO (95% CI)	P-value
Grandparents' gen	81	51.5	10.5 (6.6, 14.3)	<0.001	14	56.9	8.6 (1.7, 15.5)	0.014
Parents' gen	189	48.0	7.0 (4.2, 9.7)	<0.001	32	52.7	4.4 (-1.0, 9.8)	0.11
Probands' gen	146	41.0	0 (ref)		19	48.3	0 (ref)	
Children's gen	31	35.0	-6.0 (-10.7, 1.3)	0.013	2	37.9	-10.4 (-24.2, 3.3)	0.14

[†] mutation probability-weighted age of onset in years

Abbreviations: AoO, difference in years of age of onset; CI, confidence intervals; gen, generation; ref, reference.

Table 4

Generational and birth cohort effects in age of onset and breast and ovarian cancers

	Univariate model			Multivariable model*	
	Mean AoO †	AoO (95% CI)	P-value	Adjusted (95% CI) AoO	P-value
Breast Cancer (N=378)					
Generation					
Grandparents' gen	51.6	5.1 (0.4, 9.7)	0.033	1.8 (-3.5, 7.2)	0.51
Parents' gen	49.6	3.1 (-0.4, 6.5)	0.086	0.9 (-3.0, 4.8)	0.64
Probands' gen	46.5	0 (ref)		0 (ref)	
Children's gen	44.9	-1.6 (-14.8, 11.6)	0.81	-1.0 (-14.1, 12.0)	0.88
Birth Cohort					
1855-1919	53.5	0 (ref)		0 (ref)	
1920-1929	49.9	-3.6 (-8.3, 1.0)	0.13	-3.5 (-8.2, 1.1)	0.14
1930-1939	48.5	-5.0 (-9.4, -0.7)	0.024	-4.7 (-9.2, -0.2)	0.043
1940-1949	45.9	-7.6 (-11.9, -3.3)	0.001	-6.7 (-11.7, -1.7)	0.009
Ovarian Cancer (N=58)					
Generation					
Grandparents' gen	57.0	5.6 (-2.8, 14.0)	0.19	1.9 (-7.7, 11.4)	0.70
Parents' gen	49.6	2.1 (-4.7, 8.9)	0.54	0.3 (-7.6, 8.1)	0.95
Probands' gen	53.6	0 (ref)		0 (ref)	
Children's gen	44.2	-7.3 (-29.0, 14.4)	0.51	-7.8 (-28.9, 13.2)	0.47
Birth Cohort					
1855-1919	58.9	0 (ref)		0 (ref)	
1920-1929	57.2	-1.7 (-9.4, 6.1)	0.67	-1.2 (-9.4, 6.9)	0.76
1930-1939	48.5	-10.3 (-17.8, -2.8)	0.007	-9.7 (-17.7, -1.8)	0.016
1940-1949	53.0	-5.9 (-13.7, 2.0)	0.14	-4.3 (-13.9, 5.4)	0.39

* multivariable model include both generation and birth cohort so these two effects were adjusted each other.

† mutation probability-weighted age of onset in years

Abbreviation: AoO, difference in years of age of onset; CI, confidence intervals; ref, reference.

Table 5

Review of anticipation studies in breast cancer

Reference	N	Population	Anticipation effect for BC (years)
Jacobsen, 1946 ¹⁵	21 (m/d pairs)	Familial breast cancer	11
Morse, 1951 ¹⁴	13 (m/d pairs)	Familial breast cancer	10.5
Bucalossi, 1957 ¹³	58 (m/d pairs)	Familial breast cancer	4
Paterson, 1996 ¹⁷	59 (m/d pairs)	Familial breast cancer	6.5
Thorlacius, 1997 ²⁰	12 (m/d pairs)	<i>BRCA2</i> carriers	NSS
Paterson, 1999 ¹⁹	67 (m/d pairs)	Familial breast cancer	NSS
Dagan, 2001 ¹⁶	98 (m/d pairs)	Ashkenazi women (17 <i>BRCA1</i> ; 12 <i>BRCA2</i>)	BRCA1 – NSS BRCA2 – 18 Total – 7.18
Peixoto, 2006 ¹⁸	44 (families)	Familial breast/ovarian cancer (15 <i>BRCA1</i> ; 7 <i>BRCA2</i>)	9.5
Martinez-Delgado, 2011 ²¹	623 (m/d pairs)	Familial breast cancer (40 <i>BRCA1</i> ; 52 <i>BRCA2</i> ; <i>BRCAX</i> 531)	BRCA1 – 6.8 BRCA2 – 12.1 BRCAX – 12.3
Litton, 2012 ²²	106 (families)	<i>BRCA1/2</i> carriers	7.9
Guindalini, 2015 (current study)	176 (families)	<i>BRCA1/2</i> carriers (113 <i>BRCA1</i> ; 63 <i>BRCA2</i>)	BRCA1 – 5.8 BRCA2 – 8.4 Total – 6.8

Abbreviation: BC, breast cancer; m/d, mother/daughter; NSS, not statistically significant