Cancer variation associated with the position of the mutation in the BRCA2 gene

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Received 13 November 2003; accepted in revised form 24 November 2003

Key words: BRCA2, cancer risk, phenotypic variation

Abstract

Inherited mutations of the BRCA2 gene give rise to a multi-site cancer phenotype which includes breast cancer (in female and males), ovarian, pancreatic and prostate cancer, ocular and other melanomas, laryngeal, colon and stomach cancers. Interpretation of test results and risk assessment is therefore complex. It has been proposed that families with mutations in the ovarian cancer cluster region (OCCR) of exon 11 (nucleotides 3035–6629) express a higher ratio of ovarian to breast cancer, than families with mutations elsewhere in the BRCA2 gene. In this study we have investigated the presence of 7 types of cancer (ovary, male breast, pancreas, prostate, colon, stomach and melanoma) in first- and second-degree relatives of mutation-positive individuals in 440 families with a BRCA2 mutation. We reviewed histories of cancer in relatives among families with mutations distributed throughout the gene. Families with ovarian cancer were more likely to harbour mutations in the OCCR (nucleotides 3035–6629) than elsewhere in the gene (OR = 2.21; $P = 0.0002$). We also compared cancer risks according to ethnic group. Ashkenazi Jewish families with the 6174delT founder mutation were more likely to have a family member with ovarian cancer (OR = 1.58; $P = 0.002$) and less likely to have a family member with prostate cancer (OR = 0.62; $P = 0.04$) than were non-Jewish families. In contrast, a reduced presence of ovarian cancer was found in families of French-Canadian ancestry, compared to other ancestries (OR = 0.37; $P = 0.0026$). A high risk of male breast cancer was observed with the 6503delTT mutation (OR = 15.7; $P = 0.023$). Families of Polish ancestry had a reduced frequency of pancreatic cancer (OR = 0.0; $P = 0.03$) compared to families of other ethnic origins. In conclusion, both the position of mutation and the ethnic background of the family appear to contribute to the phenotypic variation observed in families with BRCA2 mutations.

Abbreviations: OCCR – ovarian cancer cluster region; OR – odds ratio; RR – relative risk

Introduction

The BRCA2 gene was mapped to chromosome 13q21 in 1994 [1] and was identified in 1995 [2, 3].

Subsequently, BRCA2 mutations have been identified in numerous hereditary breast cancer families. The majority are frameshift mutations and are caused by small deletions or insertions (Breast Cancer Information

Core (BIC) database) but nonsense mutations are also observed. To date it has not been proven that missense mutations in BRCA2 (which lead to single amino acid substitutions) are deleterious. Inherited mutations of the BRCA2 gene give rise to a variable cancer phenotype including female breast and ovarian cancer, male breast, pancreatic and prostate cancer, ocular and other melanomas, laryngeal, colon, stomach, gallbladder and bile duct cancers [4]. The risk is high for men as well as for women; in one study it was reported that when cancers at all sites were included, the lifetime penetrance for the development of cancer for a BRCA2 mutation for males (53%) exceeded that for females (38%) [5]. The knowledge that a particular mutation may increase the risk of a specific cancer will be of interest to members of the clinical genetics and oncology communities involved in hereditary cancer risk assessment. At-risk individuals might benefit from clinical screening for a wide spectrum of cancers.

A relatively high ratio of ovarian cancers to breast cancers was initially reported in a small study of 27 families with mutations in a 3.3 kb segment of exon 11 of BRCA2, spanned by nucleotides 3035 and 6629. Gayther et al. termed this the 'ovarian cancer cluster region' or OCCR. More recently, Thompson et al. suggested that the boundaries of the OCCR region might be narrowed slightly – the 5' boundary was mapped to the interval between nucleotides 3059–4075 and the 3' boundary was mapped to the interval between nucleotides 6503–6629 [7]. The relative risks for ovarian cancer (RR = 1.88), breast cancer (RR = 0.63) and prostate cancer (RR = 0.53) differed significantly for carriers with mutations within this region, compared to carriers with mutations in the remainder of the gene [7]. It appears therefore, that the high ratio of ovarian cancers to breast cancers in families with mutations in the OCCR is attributable to both a relatively high risk of ovarian cancer and a relatively low risk of breast cancer.

Our aim in this study was to investigate the clinical expression of mutations distributed throughout the BRCA2 gene with reference to seven different cancer types in a large collection of 440 families with BRCA2 mutations. Such data may be clinically useful in providing cancer risks to individuals harbouring BRCA2 mutations.

Materials and methods

Materials

The 440 families included in this study were selected on the basis of one or more individuals carrying a BRCA2 mutation. Pedigrees have been collected at participating research centers in Canada, the USA and Poland as part of other clinical and research protocols. The testing criteria for the families varied by center, but reports of cancers at sites other than breast or ovary

were not used to determine eligibility for testing. A variety of methods was used to identify BRCA2 mutations, including the protein truncation test (PTT), fluorescence-tagged single-strand conformation polymorphism analysis (fSSCP), denaturing gradient gel electrophoresis (DGGE), multiplex PCR for the 3 Ashkenazi Jewish founder mutations (of which BRCA2 6174delT is relevant here), multiplex PCR for the French-Canadian founder mutations (of which BRCA2 G6085T, 6503delTT and 8765delAG mutations are relevant here) and direct DNA sequencing.

The pedigree information was collated and prepared for statistical analysis at the Center for Research in Women's Health, Toronto, Canada. For each of the seven cancer types, each family was assigned to one of two categories, depending on the presence of at least one cancer of a specific type in a first- or second-degree relative of a mutation carrier in the family, or in the tested individual him/herself.

Statistical analysis

SAS version 8.1 statistical package was employed for all analyses. Fishers' exact test was used to assess statistical significance of the odds ratios. All statistical tests were two-sided.

Results

The 440 families with known BRCA2 mutations were assessed for each of 7 cancer types (ovarian, pancreatic, prostate, male breast, colon, stomach and melanoma). The ethnic distribution of the 440 families is presented in Table 1. Overall there were 140 different mutations, of which 49 were recurrent; i.e., each occurred in two or more families. The most common mutation was the

Table 1. Ethnic distribution of the 440 families.

Ethnicity	Number of families
Ashkenazi Jewish	154
Unknown	57
French-Canadian	54
British	44
German	26
Polish	26
Irish	16
Italian	17
Other White	13
Scottish	7
Filipino	5
Icelandic	4
Japanese	4
African American	3
Dutch	3
Danish	2
Hispanic	2
Greek	1
Indian	1
Iranian	1

6174delT Ashkenazi founder mutation (159 families) followed by the French-Canadian founder mutation 8765delAG (27 families). The mutations and their locations are given in Table 2. The majority of mutations were frameshifts, due to small deletions or insertions. A small proportion were nonsense mutations. The G6085T (14 families), C5910G (6 families) and C5873A (3 families) mutations accounted for the largest proportion of nonsense mutations. Missense mutations in BRCA2 have not been proven to be pathogenic and families with missense mutations were excluded. Of the 440 mutations, 331 (75.2%) were located within exon 11. Mutations in the proposed OCCR region (nucleotides 3035–6629) in exon 11 occurred in 295 families (67%).

Under the assumption of uniform distribution of cancer risk across the length of the BRCA2 gene, the observed and expected numbers of families with cancer

were calculated for each of the seven cancer types. Of particular interest are those families with mutations in the regions which can be used to help define the boundaries of the OCCR. In order to confirm or refute the results of Gayther et al. and of Thompson et al. the BRCA2 gene was divided into the following segments: A: nucleotides 1–3034; B: 3035–3058; C: 3059–4075; D: 4076–6502; E: 6503–6629 and F: 6630–9800 (Figure 1) [6, 7]. The percentage of families with ovarian cancer for families in the 5 regions are: A: 29% (18 out of 62); B: 50% (3 out of 6); C: 62% (13 out of 21); D: 50% (127 out of 254); E: 43% (6 out of 14) and F: 34% (28 out of 83). This distribution is presented graphically in Figure 1, where the odds ratio for the presence of ovarian cancer in the family, for mutations within in each 1 kb interval spanning the entire BRCA2 gene is presented. The odds ratios were below unity until approximately nucleotide 3500; were above unity

Table 2. Location of BRCA2 mutations in the 440 families.

Nucleotide change	Effect on protein	Exon	Number of families	Nucleotide change	Effect on protein	Exon	Number of families
230T>C	MIT	2	1	3772delTT	Stop 1182	11	3
250delAG	Stop 12	2	1	3782delCA	Stop 1186	11	1
254delC	Stop 24	2	2	3945delA	Stop 1258	11	1
277delAC	Stop 19	2	1	4075delGT	Stop 1285	11	7
314delTT	Stop 32	3	1	4088delA	Stop 1291	11	1
635delAC	Stop 151	4	1	4206ins4	Stop 1330	11	1
744insC	Stop 182	6	1	4265delCT	Stop 1350	11	3
886delGT	Stop 223	8	5	4304delC	Stop 1373	11	2
924delT	Stop 240	9	2	4504insA	Stop 1437	11	1
982del4	Stop 275	9	3	4643del4	Stop 1477	11	1
983del4	Stop 275	9	4	4668T>G	Y1480X	11	1
999del5	Stop 273	9	4	4677delA	Stop 1485	11	2
1184insA	Stop 326	10	1	4706del4	Stop 1502	11	3
1453delG	Stop 429	10	1	4706delGT	Stop 1502	11	1
1485delT	Stop 429	10	1	4773insA	Stop 1528	11	1
1538del4	Stop 458	10	1	4862delT	Stop 1567	11	1
1563del4	Stop 458	10	1	5008delA	Stop 1616	11	1
1917G>A	W563X	10	1	5102delAA	Stop 1637	11	1
1938del5	Stop 587	10	1	5117C>G	S1630X	11	2
1982delA	Stop 613	10	1	5163delA	Stop 1669	11	2
1983del5	Stop 587	10	1	5293delGCinsAAA	Stop 1694	11	1
2024del5	Stop 599	10	1	5301insA	Stop 1694	11	2
2034delA	Stop 613	10	2	5302insA	Stop 1694	11	2
2034insA	Stop 615	10	3	5335G>T	E1703X	11	1
2041insA	Stop 615	10	1	5445del7	Stop 1739	11	1
2041delA	Stop 613	10	1	5466insT	Stop 1747	11	1
2042insA	Stop 615	10	1	5467insT	Stop 1747	11	2
2060C>A	S611X	10	1	5482delC	Stop 1776	11	1
2157delG	Stop 659	11	2	5518delTC	Stop 1766	11	1
2816insA	Stop 880	11	3	5622C>T	R1835X	11	1
2959delG	Stop 914	11	1	5682delA	Stop 1839	11	1
3014insT	Stop 935	11	1	5699insA	Stop 1828	11	1
3034del4	Stop 958	11	11	5803delATTA	Stop 1862	11	3
3036del4	Stop 958	11	6	5804del4	Stop 1861	11	1
3271A>T	K1015X	11	1	5849del4	Stop 1907	11	4
3295insA	Stop 1025	11	1	5873C>A	S1882X	11	3
3304A>T	K1026X	11	1	5910C>G	Y1894X	11	6
3386T>G	L1053X	11	1	5946delCT	Stop 1909	11	5
3395del4	Stop 1058	11	1	5950delCT	Stop 1909	11	3
3398del5	Stop 1064	11	1	6024delTA	Stop 1943	11	1
3427delA	Stop 1076	11	2	6027del4	Stop 1961	11	1
3449del5	Stop 1078	11	1	6048del14	Stop 1944	11	1

Nucleotide change	Effect on protein	Exon	Number of families	Nucleotide change	Effect on protein	Exon	Number of families
6076delGTTA	Stop 1962	11	1	7411delC	Stop 2396	14	1
6079delAGTT	Stop 1961	11	2	7479delCA	Stop 2419	14	1
6085G>T	E1953X	11	14	7786C>T	R2520X	15	2
6092C>A	S1955X	11	2	7795delCT	Stop 2537	15	1
6137C>A	S1970X	11	2	7901delAG	Stop 2564	16	1
6158insT	Stop 2002	11	1	7989delC	Stop 2647	16	1
6174delT	Stop 2003	11	159	7990del3ins2	Stop 2647	16	1
6181delTC	Stop 2001	11	1	8136T>A	C2636X	17	1
6306delAA	Stop 2047	11	3	8138del5	Stop 2638	17	2
6468insA	Stop 2084	11	1	8237C>T	S2670L	18	1
6503delTT	Stop 2099	11	12	8377G>T	A2717S	18	1
6602insA	Stop 2128	11	1	8525delC	Stop 2776	18	2
6619insT	Stop 2131	11	1	8765delAG	Stop 2867	20	27
6633delCTTAA	Stop 2137	11	2	8803delC	Stop 2862	20	4
6659delA	Stop 2167	11	2	8864insA	Stop 2906	21	1
6673delA	Stop 2167	11	1	9076delAinsCT	Stop 2956	22	1
6674del5	Stop 2173	11	1	9132delC	Stop 2975	22	1
6696delTC	Stop 2174	11	2	9174delA	Stop 2987	22	2
6714delACAA	Stop 2166	11	2	9200del126	R2991del42	23	1
6719del4	Stop 2166	11	1	9325insA	Stop 3043	23	1
6819delTG	Stop 2201	11	1	9326insA	Stop 3043	23	1
6870delT	Stop 2228	11	1	9475insA	Stop 3110	24	1
6872delACTC	Stop 2227	11	4	9522C>A	Y3098X	25	1
6909insG	Stop 2232	11	1	9631delC	Stop 3162	25	1
6985delCT	Stop 2259	11	1	9661delGT	Stop 4048	25	1
7231del6	Stop 2337	13	1	9663delGT	Stop 3148	25	1
7297delCT	Stop 2358	14	3	9800G>A	Stop 3191	26	1

from nucleotide 3500 to 7400, and again fell below unity for nucleotides 7400 and beyond.

As in previous studies, the present analysis suggests that the OCCR does not include all of exon 11. There were 17 families with mutations in the 2157–3034 region of exon 11; i.e., 5' to the originally defined OCCR (3035–6629). The presence of ovarian cancer was significantly less for families with mutations in this region, compared to families with mutations elsewhere in BRCA2 (OR = 0.26; $P = 0.025$) or in exon 11 (OR = 0.20; $P = 0.007$). Therefore, this study supports the original assignment of Gayther et al. of the 5' border of the OCCR to be at position 3035 [6].

Originally, the 3' border of the OCCR was defined as nucleotide 6629, near the 3' boundary of exon 11. Thompson et al. propose that the 3' border of the OCCR is located in the region 6503–6629 [7]. Among the 14 families with mutations in this small interval there was neither an excess nor deficit of ovarian cancer (OR = 1.06; $P = 1.0$), therefore data are insufficient data to support a reassignment of this boundary.

In summary, this data supports the original assignment of the OCCR as encompassing nucleotides 3035 to 6629. The odds ratio for ovarian cancer being present in families with OCCR mutations, compared to non-OCCR mutations was 2.21 ($P = 0.0002$) (Table 3).

The presence of the other 6 types of cancer was determined for mutations in each of the six contiguous segments (A–F) of BRCA2, compared to mutations in the remainder of the gene. We did not confirm the observation of Thompson et al. that OCCR mutations were associated with a diminished risk of prostate cancer, although the observed association was in the right direction (OR = 0.84; $P = 0.43$) [7]. None of the other associations were significant. For no cancer type other than ovary was there a significant excess risk associated with the OCCR (Table 3).

The distribution of cancer in the families was also examined by ethnic group. There were 159 families with the 6174delT mutation in this study. Of these, 154 were reported to be Ashkenazi (or of unspecified Jewish) descent, 3 families were Polish (non-Jewish) and for 2 families ethnicity was not available. The 6174delT mutation is located within the OCCR. The risks associated with the seven cancer types were analysed for the 6174delT mutation compared to all other mutations combined (Table 4). An increased risk of ovarian cancer (OR = 1.58; $P = 0.02$) and a decreased risk of prostate cancer (OR = 0.62; $P = 0.04$) were found to be associated with the BRCA2 6174delT mutation. The association of the OCCR and ovarian cancer was not solely attributable to the 6174delT mutation. Among the other

Table 3. Comparison of cancer risk for mutations in the OCCR (3035–6629) versus remainder of the gene.

	Results			Expected values			OR	P-value
	Non-OCCR	OCCR	Total	Non-OCCR	OCCR	Total		
<i>Prostate</i>								
0	101	216	317	104.5	212.5	317	0.84	0.43
1+	44	79	123	40.5	82.5	123		
Total	145	295	440	145	295	440		
<i>Pancreas</i>								
0	126	256	382	125.9	256.1	382	1.01	1.00
1+	19	39	58	19.1	38.9	58		
Total	145	295	440	145	295	440		
<i>Ovary</i>								
0	99	146	245	80.7	164.3	245	2.20	0.0002
1+	46	149	195	64.3	130.7	195		
Total	145	295	440	145	295	440		
<i>Colon</i>								
0	114	223	337	111.1	225.9	337	1.18	0.55
1+	31	72	103	33.9	69.1	103		
Total	145	295	440	145	295	440		
<i>Male BR</i>								
0	131	261	392	129.2	262.8	392	1.22	0.63
1+	14	34	48	15.8	32.2	48		
Total	145	295	440	145	295	440		
<i>Melanoma</i>								
0	136	273	409	134.8	274.2	409	1.21	0.70
1+	9	22	31	10.2	20.8	31		
Total	145	295	440	145	295	440		
<i>Stomach</i>								
0	126	259	385	126.9	258.1	385	0.92	0.87
1+	19	36	55	18.1	36.9	55		
Total	145	295	440	145	295	440		

Table 4. Comparison of cancer risk for the 6174delT mutation *versus* mutations in the remainder of the gene.

	Results			Expected values			OR	P-value
	Other	6174	Total	Other	6174	Total		
<i>Prostate</i>								
0	193	124	317	202.4	114.6	317	0.62	0.046
1+	88	35	123	78.6	44.4	123		
Total	281	159	440	281	159	440		
<i>Pancreas</i>								
0	250	132	382	244.0	138.0	382	1.65	0.08
1+	31	27	58	37.0	21.0	58		
Total	281	159	440	281	159	440		
<i>Ovary</i>								
0	168	77	245	156.5	88.5	245	1.58	0.02
1+	113	82	195	124.5	70.5	195		
Total	281	159	440	281	159	440		
<i>Colon</i>								
0	209	128	337	215.2	121.8	337	0.70	0.16
1+	72	31	103	65.8	37.2	103		
Total	281	159	440	281	159	440		
<i>Male BR</i>								
0	250	142	392	250.3	141.7	392	0.96	1.00
1+	31	17	48	30.7	17.3	48		
Total	281	159	440	281	159	440		
<i>Melanoma</i>								
0	262	147	409	261.2	147.8	409	1.12	0.85
1+	19	12	31	19.8	11.2	31		
Total	281	159	440	281	159	440		
<i>Stomach</i>								
0	243	142	385	245.9	139.1	385	0.77	0.45
1+	37	17	55	35.1	19.9	55		
Total	281	159	440	281	159	440		

136 families with mutations in the OCCR, 6174delT excluded, the odds ratio for ovarian cancer was 2.18 ($P = 0.002$), compared to families with mutations elsewhere in the gene.

There were 54 families of French-Canadian descent. The risks were determined for the 7 cancer types in the 54 French-Canadian families compared to the remaining 386 families (Table 5). The presence of ovarian cancer was decreased in French-Canadian families, compared to other families ($OR = 0.39$, $P = 0.003$). There was no effect observed for the other six cancer types (Table 5). Among the French-Canadian families, those with a 6503delTT mutation had a higher prevalence of male breast cancer than other French-Canadian families ($OR = 15.7$; $P = 0.023$).

There were 28 families of Polish descent in the study, with BRCA2 mutations distributed throughout the gene. The Polish families showed a reduced frequency of pancreatic cancer ($OR = 0.0$; $P = 0.03$), and of colon cancer ($OR = 0.25$; $P = 0.056$) (although of borderline significance) as compared to families of other ethnic groups (Table 6).

Discussion

The results of our study of 440 families with 140 distinct BRCA2 mutations support the assignment of the OCCR to the region spanned by nucleotides 3035 and 6629. Families with mutations located within this interval were approximately twice as likely to contain women affected with ovarian cancer as families with other mutations ($OR = 2.21$; $P = 0.0002$). A relatively low risk of ovarian cancer was observed for families with mutations in the region of exon 11 encompassing nucleotides 2157–3034 ($OR = 0.26$; $P = 0.025$). This observation supports the prior assignment of the 5' border of the OCCR to nucleotide 3035. However, precise assignment of the 3' boundary of the OCCR is difficult. The risk of ovarian cancer associated with mutations in the region 6503–6619 was similar to that for other mutations ($OR = 1.06$; $P = 1.00$). The assignment of the 3' boundary of the OCCR to 6619 is somewhat arbitrary because there were relatively few families with mutations in this region. The transition in risk in the 3' boundary of the OCCR was gradual, in contrast to the 5' region for which the transition was relatively abrupt (Figure 1).

Table 5. Comparison of cancer risk and position of mutation for the French-Canadian families *versus* all other families.

	Results			Expected values			OR	P-value
	Other	F-C	Total	Other	F-C	Total		
<i>Prostate</i>								
0	283	34	317	278.1	38.9	317	0.62	0.14
1+	103	20	123	107.9	15.1	123		
Total	386	54	440	386	54	440		
<i>Pancreas</i>								
0	333	49	382	335.1	46.9	382	0.64	0.52
1+	53	5	58	50.9	7.1	58		
Total	386	54	440	386	54	440		
<i>Ovary</i>								
0	205	40	245	214.9	30.1	245	0.40	0.0035
1+	181	14	195	171.1	23.9	195		
Total	386	54	440	386	54	440		
<i>Colon</i>								
0	297	40	337	295.6	41.4	337	1.17	0.61
1+	89	14	103	90.4	12.6	103		
Total	386	54	440	386	54	440		
<i>Male BR</i>								
0	342	50	392	343.9	48.1	392	0.62	0.49
1+	44	4	48	42.1	5.9	48		
Total	386	54	440	386	54	440		
<i>Melanoma</i>								
0	339	50	409	358.8	50.2	409	1.06	0.55
1+	27	4	31	27.2	3.8	31		
Total	386	54	440	386	54	440		
<i>Stomach</i>								
0	337	48	385	337.7	47.3	385	0.85	1.00
1+	49	6	55	48.3	6.7	55		
Total	386	54	440	386	54	440		

This study provides compelling evidence that the risk of ovarian cancer in all BRCA2 families is not uniform – but it is nevertheless difficult to generate precise estimates of risk for individuals based on the position of the mutation in the family. There are several limitations to our study which preclude a definitive analysis. The cancers documented in the families were based on proband interviews and the majority of reported cancers were not confirmed. It is possible that some cancers were missed and others were misassigned. Ideally, we are interested in only those cancers in BRCA2 carrier relatives, and it is expected that only one-half of the first-degree relatives will carry the mutation that is identified in the proband. We did not know the mutation status for the affected relatives. Families were classified into those with and without a cancer of a particular site. To the extent that family sizes vary and differences in the diligence applied in obtaining pedigree details, the probability of ascertaining cancers in relatives will vary. The entry criteria for testing families for BRCA2 mutations may vary from center to center. This will create a problem if a cancer of interest is used as a criteria for testing at one center but not at another. However, it is unlikely that cancers other than of the breast and ovary

were used in pedigree ascertainment and testing decisions. Rates of prostate cancer are highly dependent on screening practices and the observed variation in rates between families and between countries may reflect differences in the utilization of screening. For other sites, such as ovary and pancreas, screening is less of a concern. Finally, multiple comparisons were made and many of the associations reported here are consistent with chance variation. The ideal study would be to follow prospectively a large cohort of healthy BRCA2 carriers for all incident cases of cancer. However, in order to generate accurate risk estimates for individual cancer types and for different classes of mutations it would be necessary to follow several thousand mutation carriers for an extended period of time.

Ultimately, it is hoped that knowledge of genotype/phenotype correlations will allow genetic counselors to provide mutation-specific risk estimates for the different forms of cancer. With the exception of ovarian cancer, the data is not yet sufficiently strong to reject the prior hypothesis that the risk is uniform across the gene, and it is important that our observations be extended. In the case of ovarian cancer, it appears that mutations in the 3' portion of exon 11 confer the highest risk of ovarian

Table 6. Comparison of cancer risk and position of mutation for the Polish families *versus* all other families.

	Results			Expected values			OR	P-value
	Other	Polish	Total	Other	Polish	Total		
<i>Prostate</i>								
0	294	23	317	298.3	18.7	317	0.32	0.07
1+	120	3	123	115.7	7.3	123		
Total	414	26	440	414	26	440		
<i>Pancreas</i>								
0	356	26	382	359.4	22.6	382	0.00	0.03
1+	58	0	58	54.6	3.4	58		
Total	414	26	440	414	26	440		
<i>Ovary</i>								
0	231	14	245	230.5	14.5	245	0.92	0.84
1+	183	12	195	183.5	11.5	195		
Total	414	26	440	414	26	440		
<i>Colon</i>								
0	313	24	337	317.1	19.9	337	0.26	0.056
1+	101	2	103	90.4	6.1	103		
Total	414	26	440	414	26	440		
<i>Male BR</i>								
0	366	26	392	368.8	23.2	392	0.00	0.09
1+	48	0	48	45.2	2.8	48		
Total	414	26	440	414	26	440		
<i>Melanoma</i>								
0	383	26	409	384.8	24.2	409	0.00	0.24
1+	31	0	31	29.2	1.8	31		
Total	414	26	440	414	26	440		
<i>Stomach</i>								
0	365	20	385	362.3	22.7	385	2.23	0.12
1+	49	6	55	51.7	3.3	55		
Total	414	26	440	414	26	440		

cancer. We also saw an increase in ovarian cancer risk with mutations in the 3' region of the gene, encompassing exons 23–26 (Figure 1) but this peak was based on a small number of families and did not reach statistical significance.

The present study does not permit us to estimate lifetime penetrance rates of ovarian cancer for women with BRCA2 mutations. Knowledge of age-specific ovarian cancer risks would be useful for women and their physicians in making decisions regarding preventive oophorectomy. Oral contraceptives are an attractive option for BRCA2 carriers as they have been shown to diminish the risk of ovarian cancer [8], and appear not to increase the risk of breast cancer [9]. Therefore it is reasonable to offer an oral contraceptive to BRCA2 carriers at all levels of risk.

This study is the first to consider the variation in cancer risk among BRCA2 carriers associated with the different ethnic groups. Because the distribution of mutations differs among different ethnic groups (primarily due to the presence of common founder mutations) it is difficult to distinguish the effects of the mutation from that of the ethnic group. For example, Jewish women with the 6174delT mutation comprised 35% of all families studied. There were no Jewish women with other mutations and only three women with

the 6174delT mutations reported themselves to be non-Jewish. Therefore it is not possible to distinguish the effect of Jewish ancestry from that of the specific 6174delT mutation.

Our data suggests the possibility of a colon cancer (OR = 3.57; $P = 0.0072$) and stomach cancer (OR = 2.82; $P = 0.043$) cluster region bounded by nucleotides 3059–4075, but this needs to be confirmed. The observed differences in cancer rates in BRCA2 carriers may reflect local cancer incidence rates [10]. This difference appears to be present among BRCA2 carriers as well. Jakubowska et al. previously noted a high incidence of stomach cancers in Polish families with BRCA2 mutations [11]. The relative risk for stomach cancer among BRCA2 carriers compared to non-carriers has been estimated to be 2.6 [4].

The cumulative risk of developing male breast cancer by age 80 years in a BRCA2 carrier has been estimated to be 7% [7]. We found evidence of male breast cancer risk being significantly greater than expected for French-Canadian families with the 6503delTT mutation (OR = 15.7; $P = 0.02$); however, this observation was based on a few families. French-Canadian families were found to have fewer ovarian cancers compared to other families (OR = 0.37; $P = 0.003$). It appears that ovarian cancer risk is dependent on both ethnic background and

mutation position. The ethnic association may be due to modifying genes or to the local prevalence of selected reproductive risk factors, such as oral contraceptive use, parity, breastfeeding and oophorectomy.

Pancreatic cancer is the fourth leading cause of death due to cancer for both males and females in the USA and cases of pancreatic cancer have been observed in breast-ovarian cancer families with BRCA2 mutations [12, 13]. The Breast Cancer Linkage Consortium estimates the relative risk for pancreatic cancer among BRCA2 carriers to be 3.5 [4]. Fifty-eight families in this study had at least one case of pancreatic cancer, but we saw no evidence that the position of the mutation in the gene has an influence on pancreatic cancer risk. However, there was no pancreatic cancer in the 28 Polish families in this study, whose mutations were distributed throughout the gene. Melanomas have also been observed in breast-ovarian cancer families with mutations in BRCA2. The relative risk for developing malignant melanoma has been estimated to be 2.6 [4]. In particular, ocular melanomas have been observed at a higher frequency than expected in BRCA2 carriers [14, 15]. In our study, 31 of 440 families reported melanoma, but there was no evidence that the position of the BRCA2 mutation influenced melanoma risk.

The biological explanation for the variation in risk is unclear. BRCA2 functions in the maintenance of genomic stability and in the repair of double-strand DNA breaks [16–18]. Interestingly, the OCCR is coded for by the large exon 11 and overlaps with the region of exon 11 which contains 8 BRC repeat units. These repeats are globular structural motifs that mediate binding to RAD51, the human homolog of bacterial RecA. RAD51 is involved in directing strand invasion during recombination [19–21]. BRCA2 regulates RAD51 function by controlling its intracellular localization and by modulating its ability to form nucleofilaments. The latter function is mediated by two of the BRC repeats, BRC3 and BRC4 [22–25]. Located 5' of the BRC repeats is a region which shares high identity with other BRCA2 orthologues (and is likely to be functionally important) [26]. This region interacts with DSS1 and is able to bind single-stranded DNA [26–27]. The carboxy-terminal region of BRCA2 also includes the nuclear localization signals (NLS) [28].

Assuming that premature truncated proteins are generated and are stable, we might speculate that the loss of the RAD51- and DSS1-binding regions, alone or in combination, might explain the observed variation in ovarian cancer risk. The data in Figure 1 show a drop in the odds ratio that coincides with the loss of the DSS1-interacting region. Partial length transcripts which contain this region will invariably disrupt the DSS1-interacting region and the NLS but will retain the RAD51-binding region. Similarly, mutations which retain all but the N-terminal region are associated with a low risk of ovarian cancer. The increase in ovarian cancer risk coincides with the retention of the BRC repeat clusters. Mutations in the N-terminal region will

result in proteins which have lost all the known structural domains of BRCA2 (NLS, RAD51- and DSS1-interacting region). Conversely, mutations in the region presenting the highest risk of ovarian cancer are expected to disrupt the DSS1-interacting region and the NLS, but still retain some or all BRC repeats.

The inspection of the BRCA2 functional domains does not help to explain why the regions of high risk for ovary and breast cancer should be different. One possibility is that different mutations have different effects on the apoptosis response in the breast and ovary. Conceivably, a mutation could trigger early apoptosis in the ovary but not in the breast and therefore the breast cells could progress through tumorigenesis. An independent line of research suggests that mutations that lead to transcripts which retain the RAD51-binding region generate a less severe phenotype than mutations located in the 5' region of the protein. Recently, it was shown that some patients with Fanconi anemia carry biallelic inactivation of BRCA2. Germline biallelic inactivation of BRCA2 had previously been considered to be embryonic lethal suggesting that the mutations associated with Fanconi anemia have a less severe clinical expression. Fanconi anemia patients carrying mutations in BRCA2 have either both or at least one allele with truncations located 3' to exon 11 [29]. Individuals with truncating mutations in exon 27 and which lead to the loss of the terminal portion of the BRCA2 gene seem to have no functional consequence in the heterozygous state [30] but the possibility of a homozygote effect has not been excluded.

Alternatively, differences in risk could be explained by differential mRNA degradation mediated by the nonsense-mediated decay (NMD) pathway. It has been proposed that NMD is triggered by premature termination codons (PTC) which are situated close to an exon-exon junction. Interestingly, it has been shown that PTC located more than 559 nucleotides 5' to the exon-exon junction escaped NMD detection [31]. In this case mutations in the large exon 11 of BRCA2 would have a higher chance of escaping NMD-mediated degradation than mutations in the small exons, which are all in the vicinity of an exon-exon boundary. Mutations in the OCCR would therefore be more likely to lead to the production of a protein product than mutations elsewhere in the gene. However, a recent study on PTC in BRCA1, which also contains a large exon 11, showed that PTC located farther than 559 nucleotides from an exon-exon boundary were still subject to NMD [32]. It remains to be seen if this is also the case for BRCA2.

Conclusions

In conclusion, the risk of ovarian cancer among carriers of BRCA2 mutations varies according to the position of mutation in gene. Additional factors which modify cancer risk include country of origin, which may in turn

reflect differences in ethnic background and in environmental and lifestyle risk factors. The biological basis for the differences in cancer risk remain to be explored. It is not yet possible to offer specific ovarian cancer risk estimates to patients with different mutations in the clinical setting.

Acknowledgements

We would like to thank the many clinicians and genetic counsellors who contributed families to this study, including Olufunmilayo Olopade, Howard Saal, Donna Gichrist, Beth Karlan, Peter Ainsworth, Albert Chudley, Sofia Merajver, Gordon Mills, David Fishman, Charis Eng, Ed Lemire, and William Foulkes. Supported by the Canadian Genetic Diseases Network, the Canadian Breast Cancer Research Foundation (Ontario chapter) and the Canadian Breast Cancer Research Initiative. CMP is funded by US Army grant DAMD17-00-1-0478.

References

1. Wooster R, Neuhausen S, Mangion J et al. Localisation of a breast cancer susceptibility gene (BRCA2) to chromosome 13q by genetic linkage analysis. *Science* 1994; 265: 2088–90.
2. Wooster R, Bignell G, Swift S et al. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995; 378: 789–92.
3. Tavtigian SV, Simard J, Rommens J et al. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nat Genet* 1996; 12: 333–7.
4. BCLC – The Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst* 1991; 91: 1310–6.
5. Risch HA, McLaughlin JR, Cole DE et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. *Am J Hum Genet* 2001; 68: 700–10.
6. Gayther SA, Mangion J, Russell P et al. Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. *Nat Genet* 1997; 15: 103–5.
7. Thompson D, Easton D. Breast Cancer Linkage Consortium. Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. *Am J Hum Genet* 2001; 68: 410–9.
8. Narod SA, Risch H, Moslehi R et al. Oral contraceptives and the risk of hereditary ovarian cancer. *N Engl J Med* 1998; 339: 424–8.
9. Narod SA, Dube M-P, Klijn J et al. Oral contraceptives and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *J Natl Cancer Inst* 2002; 94: 1773–9.
10. Parkin DM, Whelan SL, Ferlay J et al. *Cancer Incidence in Five Continents*. Lyon: IARC Press, 1997.
11. Jakubowska A, Nej K, Huzarski T et al. BRCA2 gene mutations in families with aggregations of breast and stomach cancers. *Br J Cancer* 2002; 87: 888–91.
12. Phelan CM, Lancaster JM, Tonin P et al. Mutation analysis of the BRCA2 gene in 49 site-specific breast cancer families. *Nat Genet* 1996; 13: 120–2.
13. Moslehi R, Chu W, Karlan B et al. BRCA1 and BRCA2 mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. *Am J Hum Genet* 2000; 66: 1259–72.
14. Sinilnikova OM, Egan KM, Quinn JL et al. Germline BRCA2 sequence variants in patients with ocular melanoma. *Int J Cancer* 1999; 82: 325–8.
15. Iscovich J, Abdulrazik M, Cour C et al. Prevalence of the BRCA2 6174delT mutation in Israeli uveal melanoma patients. *Int J Cancer* 2002; 98: 42–4.
16. Scully R, Livingston D. In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature* 2000; 408: 429–32.
17. Venkitaraman AR. Cancer Susceptibility and the Functions of BRCA1 and BRCA2. *Cell* 2002; 108: 171–82.
18. Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol Cell* 2001; 7: 263–72.
19. Bork P, Blomberg N, Nilges M. Internal repeats in the BRCA2 protein sequence. *Nat Genet* 1996; 13: 22–3.
20. Chen PL, Chen CF, Chen YM et al. The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methane-sulfonate treatment. *Proc Natl Acad Sci USA* 1998; 95: 5287–92.
21. Katagiri T, Saito H, Shinohara A et al. Multiple possible sites of BRCA2 interacting with DNA repair protein RAD51. *Genes Chromosomes Cancer* 1998; 21: 217–22.
22. Chen CF, Chen PL, Zhong Q et al. Expression of BRC repeats in breast cancer cells disrupts the BRCA2-Rad51 complex and leads to radiation hypersensitivity and loss of G(2)/M checkpoint control. *J Biol Chem* 1999; 274: 32931–5.
23. Davies AA, Masson JY, McIlwraith MJ et al. Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. *Mol Cell* 2001; 7: 273–82.
24. Xia F, Taghian DG, DeFrank JS et al. Deficiency of human BRCA2 leads to impaired homologous recombination but maintains normal nonhomologous end joining. *Proc Natl Acad Sci USA* 2001; 98: 8644–9.
25. Stark JM, Hu P, Pierce AJ et al. ATP hydrolysis by mammalian RAD51 has a key role during homology-directed DNA repair. *J Biol Chem* 2002; 277: 20185–94.
26. Yang HJ, Jeffrey PD, Miller J et al. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* 2002; 297: 1837–48.
27. Marston NJ, Richards WJ, Hughes D et al. Interaction between the product of the breast cancer susceptibility gene BRCA2 and DSS1, a protein functionally conserved from yeast to mammals. *Mol Cell Biol* 1999; 19: 4633–42.
28. Spain BH, Larson CJ, Shihabuddin LS et al. Truncated BRCA2 is cytoplasmic: Implications for cancer-linked mutations. *Proc Natl Acad Sci USA* 1999; 96: 13920–5.
29. Howlett NG, Taniguchi T, Olson S et al. Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* 2002; 297: 606–9.
30. Mazoyer S, Dunning AM, Serova O et al. A polymorphic stop codon in BRCA2. *Nat Genet* 1996; 14: 253–4.
31. Zhang J, Sun XL, Qian YM et al. At least one intron is required for the nonsense-mediated decay of triosephosphate isomerase mRNA: A possible link between nuclear splicing and cytoplasmic translation. *Mol Cell Biol* 2002; 18: 5272–83.
32. Perrin-Vidoz L, Sinilnikova OM, Stoppa-Lyonet D et al. The non-sense mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature stop codons. *Hum Mol Genet* 2002; 11: 2805–14.