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Panel Testing for Familial Breast Cancer: Calibrating the Tension Between Research and Clinical Care

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ABSTRACT

Purpose
Gene panel sequencing is revolutionizing germline risk assessment for hereditary breast cancer. Despite scant evidence supporting the role of many of these genes in breast cancer predisposition, results are often reported to families as the definitive explanation for their family history. We assessed the frequency of mutations in 18 genes included in hereditary breast cancer panels among index cases from families with breast cancer and matched population controls.

Patients and Methods
Cases (n = 2,000) were predominantly breast cancer-affected women referred to specialized Familial Cancer Centers on the basis of a strong family history of breast cancer and *BRCA1* and *BRCA2* wild type. Controls (n = 1,997) were cancer-free women from the LifePool study. Sequencing data were filtered for known pathogenic or novel loss-of-function mutations.

Results
Excluding 19 mutations identified in *BRCA1* and *BRCA2* among the cases and controls, a total of 78 cases (3.9%) and 33 controls (1.6%) were found to carry potentially actionable mutations. A significant excess of mutations was only observed for *PALB2* (26 cases, four controls) and *TP53* (five cases, zero controls), whereas no mutations were identified in *STK11*. Among the remaining genes, loss-of-function mutations were rare, with similar frequency between cases and controls.

Conclusion
The frequency of mutations in most breast cancer panel genes among individuals selected for possible hereditary breast cancer is low and, in many cases, similar or even lower than that observed among cancer-free population controls. Although multigene panels can significantly aid in cancer risk management and expedite clinical translation of new genes, they equally have the potential to provide clinical misinformation and harm at the individual level if the data are not interpreted cautiously.

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INTRODUCTION

Clinical genetic testing for hereditary cancer syndromes has been transformed by the advent of massively parallel sequencing, which allows simultaneous screening of a large number of genes at a fraction of the cost previously required to sequence just one gene (such as *BRCA1*).[1] Although the technical efficiency afforded by panel gene tests in providing more comprehensive genomic interrogation is attractive, the temptation to include more speculative genes for which there are limited data relating to cancer risk is of concern.[2] This trend has led to the development of commercial breast cancer multigene panels that are being used in the clinical diagnostic setting but provide sequence data relating to genes of unknown clinical significance, more in keeping with a research study.

Ironically, the high cost and other disadvantages of single-gene testing previously ensured that only those genes with confident penetrance estimates and clear clinical utility were sequenced and the results reported back to patients. Of particular concern with the increasing use of these panel tests for hereditary breast and ovarian cancer predisposition in the diagnostic setting is the absence of data for matched population controls to inform the interpretation of the resulting data. Exome sequencing of *BRCAX* families for new breast cancer predisposition genes demonstrates that loss-of-function (LoF) mutations in genes that are plausibly involved in cancer

predisposition, based on their known or presumed cellular functions, are common.[3] In a diagnostic context, interpreting the relevance of LoF mutations in genes of unknown clinical significance, which may vary widely in frequency across different populations, becomes highly challenging.[4]

To assess the clinical value of panel testing in the hereditary breast cancer setting, we analyzed the performance matrix of 18 known or proposed breast cancer predisposition genes, selected from those included in commercially available diagnostic multigene panel tests (Table 1), in 2,000 women affected by breast cancer, enriched for features of a hereditary basis, but previously screened and found to be negative for *BRCA1* and *BRCA2* mutations, and 1,997 cancer-free controls.

PATIENTS AND METHODS

Patient Cohorts

A total of 999 breast cancer or ovarian cancer-affected index cases (95% of patients had a primary diagnosis of breast cancer) were ascertained from the Hunter Area Pathology Service, Newcastle, Australia[5] and 1,001 breast cancer-affected index cases from the Variants in Practice Study from the combined Familial Cancer Centers, Melbourne, Australia.[6] For all cases, the personal and/or family history was determined by a provider trained in cancer genetics to be sufficiently strong to be eligible for clinical genetic testing. Cases had been referred for clinical genetic testing between 1997 and 2014 but had tested negative for pathogenic mutations in *BRCA1* and *BRCA2* using the test technology routinely available at that time. The overall detection rate for *BRCA1* and *BRCA2* mutations in women meeting these criteria who were referred to the Familial Cancer Centers over this period has varied between 17% and 20%.

The control population included 1,997 participants in the LifePool study[6a] who were cancer free at last follow-up (January 2015). LifePool predominantly recruited women who were older than 40 years of age through the population-based mammographic screening program in the state of Victoria, Australia (BreastScreen Victoria). The average age of LifePool participants was 59.9 (standard deviation, 9.9) years (range, 40-92 years). All participants gave informed consent for genetic testing and the study was approved by the Human Research Ethics Committees at all participating centers.

Panel-Based Variant Screening

Germline DNA from blood (all cases and 97.4% of controls) or saliva (2.6% of controls) was analyzed using a custom targeted sequencing panel of 18 genes that are included on at least one commercially available breast cancer predisposition panel test (Appendix Table A1, online only).[2] The panel included all exons of *ATM*, *ATR*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *MRE11A*, *NBN*, *NF1*, *PALB2*, *PTEN*, *RAD50*, *STK11*, *TP53*, and *XRCC2*. All exons and at least 10 bp of intronic sequence were targeted using the HaloPlex Targeted Enrichment Assay (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol. Indexed, 96-sample pooled libraries were analyzed on a HiSeq2500 Genome Analyzer (Illumina, San Diego, CA) to generate paired-end 100- or 150-bp sequencing reads. Sequencing alignment was performed using the Burrows-Wheeler Alignment tool,[7] and base quality score recalibration and indel realignment were performed using the Genome Analysis Toolkit.[8] Variant calling was undertaken using the Genome Analysis Toolkit UnifiedGenotyper v2.4 (Broad Institute, Cambridge, MA).[9]

As with most gene panel sequencing assays, current alignment tools do not reliably identify deletions or insertions larger than 15 to 20 bp. Variants were described according to Human Genome Variation Society guidelines.[9a] The variant data were filtered to identify all protein-truncating LoF mutations, which include nonsense, frameshift, stop gain, stop lost, and essential splice-site mutations. In addition, due to limitations in methods for classification of nonsynonymous variants and to replicate what is happening in many genetics services, only those nonsynonymous variants unequivocally assigned as pathogenic by ClinVar[10] were included in the analysis. All LoF and pathogenic nonsynonymous mutations were validated by Sanger sequencing.

Odds ratios and *P* values by two-tailed Fisher's exact tests were calculated using GraphPad Prism software (GraphPad Software, San Diego, CA).

Table 1. Frequency of Pathogenic Mutations in Panel Genes

	Cases (n = 2,000)			Controls (n = 1,997)				
Gene	LoF	Pathogenic Missense	Total (% carrier frequency)	LoF	Pathogenic Missense	Total (% carrier frequency)	*P**	OR (95% CI)
BRCA1	2	2	4	4	0	4 (0.20)	NA	NA
BRCA2	1	1	2	9	0	9 (0.45)	NA	NA
ATM	8	1	9 (0.45)	4	0	4 (0.20)	.27	2.15 (0.692 to 7.33)
ATR	3	0	3 (0.15)	1	0	1 (0.05)	.37	3.03 (0.31 to 29.1)
BARD1	3	0	3 (0.15)	1	0	1 (0.05)	.62	3 (0.312 to 28.9)
BLM	5	0	5 (0.25)	3	0	3 (0.15)	.73	1.67 (0.397 to 6.98)
BRIP1	7	0	7 (0.35)	4	0	4 (0.20)	.55	1.75 (0.511 to 5.99)
CDH1	1	0	1 (0.05)	0	0	0	.50	3.01 (0.12 to 74.1)
CHEK2	2	6	8 (0.40)	0	6	6 (0.3)	.79	1.33 (0.461 to 3.85)
MRE11A	4	0	4 (0.20)	0	0	0	.12	9.0 (0.484 to 167)
NBN	2	0	2 (0.1)	3	0	3 (0.15)	1.00	0.67 (0.11 to 4.0)
NF1	1	0	1 (0.05)	1	0	1 (0.05)	1.00	1.0 (0.06 to 16)
PALB2	26	0	26 (1.3)	4	0	4 (0.20)	< .001	6.56 (2.29 to 18.8)
PTEN	1	0	1 (0.05)	0	0	0	.50	3.01 (0.12 to 74.1)
RAD50	2	0	2 (0.1)	6	0	6 (0.3)	.45	0.50 (0.0912 to 2.73)
STK11	0	0	0	0	0	0	NA	NA
TP53	1	4	5 (0.25)	0	0	0	.03	11 (0.61 to 201)
XRCC2	2	0	2 (0.1)	0	0	0	.25	5.05 (0.24 to 105)

Abbreviations: LoF, loss of function; NA, not applicable; OR, odds ratio.
*Two-sided Fisher's exact test.

RESULTS

Sequencing of 3,997 samples was performed, with an average of 94% of the coding regions covered by at least 10 reads (93% for cases and 95% for controls). A detailed list of specific variants is given in Appendix Table A2 (online only). Six case individuals were found to harbor actionable mutations in *BRCA1* or *BRCA2* as defined in Patients and Methods. These cases presented when full *BRCA1* and *BRCA2* diagnostic sequencing was not available and these mutations would have been detected with the clinical testing technology used now (Appendix Table A2). The frequency of pathogenic mutations in *BRCA1* and *BRCA2* in the control group was 0.2% (four mutations) and 0.45% (nine mutations), respectively (Table 1; Appendix Table A2), which is consistent with previous indirect estimates for European populations.[11] However, to our knowledge, this is the largest direct assessment of their prevalence. The *BRCA1/2*-positive individuals were not included in the further analysis.

In the remaining participants with no *BRCA1* or *BRCA2* mutation, a total of 79 variants in 1,994 cancer cases and 33 variants in 1,984 control individuals met the definition of an actionable mutation to give an overall sensitivity of 4% (95% CI, 3.2% to 4.9%). LoF mutations identified, as well as known pathogenic missense variants, are summarized in Table 1. The greatest contributor to the detection rate was *PALB2*, where 26 protein-truncating mutations were detected among cases versus four among the controls ($P < .001$), in keeping with the approximately 1% prevalence previously described in familial breast cancer.[11,12] Five cases were found to carry pathogenic *TP53* variants. On review, none of these individuals had a family history that meets the classic criteria for a diagnosis of Li-Fraumeni syndrome. The mutation detection rate stratified by age did not differ markedly in the cases or controls (Table 2).

The presence of a significant number of actionable mutations in the control group means that the positive predictive value of an abnormal finding is curtailed at 0.71 (95% CI, 0.61 to 0.79). Of note, almost half of the controls are older than 60 years old and only 13% are younger than 50 years old and therefore should be somewhat depleted for strongly cancer-causing mutations relative to the cases. On the basis of these data, we estimate that the contribution of mutations in these 16 genes (excluding *BRCA1* and *BRCA2*) to the incidence of breast cancer (the population attributable risk) as a maximum of 2.3%, although the highly selected nature of the case group means the figure for the general population will be less than this. Approximately half of the estimated population attributable risk is due to mutations in *PALB2* alone.

Beyond *TP53* and *PALB2*, the contribution of the other genes in the panel was modest. Mutations were not detected in *STK11* in either the cases or controls. Among the remaining 13 genes, the mutation frequency was low, with only 48 cases (2.4%) carrying previously reported pathogenic or novel LoF mutations compared with 29 (1.5%) among the controls. For most of these genes, there was a slight excess of putative mutations in the case group consistent with a moderate risk (combined odds ratio [OR], 1.67; 95% CI, 1.05 to 2.65), but the effect was inconsistent. For example, for *NBN* and *RAD50*, there were more mutations detected among the controls than the cases, whereas the case and control frequencies were similar for *CHEK2* and *ATM*. Only the excess of mutations in cases for *PALB2* and *TP53* were statistically significant compared with controls. Our study failed to provide additional support for a role of the other genes included in the panel as high-risk breast cancer predisposition genes, and it was underpowered to detect effects for rarely mutated genes, such as *CDH1* and *STK11*, or the possibility of variant-specific high-risk effects. Our overall frequency of actionable mutations may be underestimated because we adopted a conservative approach to interpretation of nonsynonymous variants to replicate what is happening in many genetics services. In addition, we cannot exclude the possibility that there may be enrichment among the cases of mutation classes not detectable by this specific assay, such as copy number or structural variations.

Table 2. Mutation Detection Frequency According to Age

Age, Years‡	Cases (n = 1,994)*		Controls (n = 1,997)†	
	Total No. (%)	No. With Actionable Mutations (%)	Total No. (%)	No. With Actionable Mutations (%)
< 40	456 (22.9)	17 (3.73)	0 (0)	0
40-44	286 (14.3)	11 (3.85)	66 (3.3)	1 (1.52)
45-49	316 (15.8)	14 (4.43)	202 (10.1)	6 (2.97)
50-54	261 (13.1)	15 (5.75)	415 (20.8)	14 (3.37)
55-59	243 (12.2)	6 (2.47)	351 (17.6)	7 (1.99)
≥ 60	432 (21.7)	16 (3.70)	963 (48.2)	18 (1.87)
Totals	1,994	79	1,997	46

*Number of cases excludes the six individuals subsequently found to carry pathogenic *BRCA1* or *BRCA2* mutations.
†Number of controls includes the 13 individuals with pathogenic *BRCA1* or *BRCA2* mutations.
‡Age at diagnosis for the cases and age at collection for the controls.

DISCUSSION

There is an increasing demand by both health care providers and patients for panel testing for inherited syndromes due to the perceived clinical and economic advantages of this approach. In particular, numerous groups have recently reported variant data from breast cancer predisposition gene panels in individuals who have been clinically assessed as having a strong family history of breast cancer.[12-14] In general, these studies report the frequency of genes with actionable mutations based solely on whether the variant is likely to cause a loss of protein function, with no reference to either the local population frequency of such mutations or the strength of the data supporting a breast cancer predisposing role for that particular gene.

In this study, when applied to a large group of women affected by breast cancer with the highest risk of carrying a clear genetic cause, the use of a multigene panel in a research setting has provided important clinical information to 34 families: 26 with a pathogenic *PALB2* mutation, five with a *TP53* mutation, one with a *CDH1* mutation, one with a *PTEN* mutation, and one with a well-established pathogenic missense mutation in *ATM* (c.7271T>G). These findings emphasize the benefit of being able to offer more extensive screening of genes with established clinical utility, even if

mutations in these genes are extremely rare. For other genes, the data are conflicting, equivocal, or only convincing for specific variants in a gene. For example, although the *ATM* (c.7271T>G) missense mutation has been clearly established as a high-risk allele,[15] truncating *ATM* mutations may confer only a modest risk; this is supported by our finding of similar numbers among the cases and controls (8 *v* 4; $P = .27$). This is not due to bias in the control group because the carrier frequency of *ATM* LoF mutations reported in the Exome Aggregation Consortium database[15a] is 0.28% compared with 0.20% among LifePool participants.

The data supporting a role for *RAD50* in breast cancer are particularly speculative. Originally identified as a potential breast cancer gene in a Finnish population,[16] subsequent studies have been equivocal at best.[17] Our data do not support a role for *RAD50* in breast cancer predisposition, with more truncating mutations identified in the controls than the cases (2 *v* 6; $P = .45$). This is consistent with a recent study of over 1,200 cases and 1,100 controls, which failed to identify a significant excess of either truncating (OR, 1.16; 95% CI, 0.26 to 5.18) or rare missense variants (OR, 3.21; 95% CI, 0.68 to 15.2).[17] In contrast, recent studies have clearly established *PALB2* as a moderate to high penetrance breast cancer predisposition gene,[18] and this is mirrored by our detection of a highly significant excess of mutations in this gene among cases ($P < .001$). *PALB2* is perhaps now the leading example of an ideal panel gene, where the association of LoF mutations with a clinically significant risk is clear but where the frequency of these mutations, even in women with a strong family history, is so low that screening will always be most efficient as part of a panel.

Had this multigene panel test been offered to the cases as a diagnostic test in the clinical setting, 79 non–*BRCA*-related pathogenic or novel LoF mutations would have been identified. Of these, only 34 (43%) involved genes for which evidence-based cancer risk-management guidelines are sufficiently developed to allow accurate targeted genetic counseling and breast cancer risk management (*PALB2*, *TP53*, *CDH1*, *STK11*, *PTEN*, and the *ATM* c.7271T>G mutation). Cascade testing within the families for the remaining 45 (57%) variants has potential for clinical disinformation and implementation of inappropriate risk-management strategies. Following this study, we have offered confirmatory testing in the clinical setting to the study participants for whom established pathogenic variants were detected in *BRCA1*, *BRCA2*, *TP53*, *PALB2*, *CDH1*, *PTEN*, and the *ATM* c.7271T>G mutation.

RAD51C and *RAD51D* have been identified as ovarian and potentially breast cancer predisposing genes[19,20] and are now included on most commercial breast cancer panels. However, convincing evidence supporting a breast cancer-predisposing role for these genes was not available during our initial panel design phase; thus, data are only available for 997 cases and 983 controls. LoF mutations were rare in both *RAD51C* (zero cases, one control) and *RAD51D* (one case, one control).

A key impetus for including as many genes as possible on panels is likely to be economic, because there are no longer major cost constraints to testing multiple genes. However, the demand for more extensive genetic testing through increasing technological improvements should not compromise the obligation to apply the robust principles of test development that have been established.[20a] Our data, along with the current state of evidence from other studies and as reviewed recently by Easton et al,[2] indicate that many of the genes included in the current commercial panels do not conform to current guidelines. Nevertheless, with informed consent, prudent use of data from extended panel testing can expedite the clinical translation of new genetic findings.

Indeed, this approach could be pivotal in providing diagnostic levels of confidence in penetrance estimates for a large number of rare variants in a markedly quicker time scale than previous approaches. Operating in this new paradigm, where the boundaries between research and practice are less distinct, requires the formulation of robust rules regarding levels of evidence for clinical translation.[21] This is now a matter of some urgency because consumers are already able to independently purchase commercially available breast cancer panel tests. Harnessing the research potential of all the clinical panel testing currently being undertaken may be possible through collaboration among diagnostic testing laboratories or through innovations such as the Prospective Registry of Multiplex Testing registry,[22] where consumers can be encouraged (with the support of many commercial laboratories) to upload their own panel test results, either with or without additional clinical information.

Our data show that attributing a causative role for mutations identified in many genes commonly included on breast cancer predisposition panels is complex, involving variant-, gene-, and population-specific characteristics. Although we support the use of multigene panels in the clinical setting as a powerful opportunity to produce large pragmatic genomic data sets, at the individual level this information needs to be interpreted with caution to avoid the potential to provide clinical misinformation and cause harm.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

Table A1. Genes Included on Commercial Breast Cancer Panels to Assess Breast Cancer Risk as Described by Provider

Gene	This study	Women's Hereditary Cancers, Breast/Invitae	BROCA Panel, Breast/University of Washington	myRisk, Breast/ Myriad	BreastNext/ Ambry	HBOC and HBOC Expanded/ Prevention Genetics	Breast and Ovarian Cancer: Breast Cancer Risk/GeneDX	Hereditary Breast, Ovarian, Endometrial/ Baylor College of Medicine	High-Risk Breast Cancer/Emory Genetics	Breast Ovarian Cancer NGS Panel/Fulgent Diagnostics
ATM	+	+	+	+	+	+	+	+		+
AKT1			+							
ATR	+									+
BARD1	+	+	+	+	+		+	+		+
BLM	+									+
BRCA1	+	+	+	+	+	+	+	+	+	+
BRCA2	+	+	+	+	+	+	+	+	+	+
BRIP1	+	+	+	+	+	+	+	+		+
CDH1	+	+	+	+	+	+	+	+	+	+
CHEK2	+	+	+	+	+	+	+	+		+
FAM175A			+							
FANCC		+					+			+
GEN1			+							
MRE11A	+	+	+	+	+	+		+		+
NBN	+	+	+	+	+	+	+	+		+
NF1	+	+	+		+					
PALB2	+	+	+	+	+	+	+	+	+	+
PTEN	+	+	+	+	+	+	+	+	+	+
RAD50	+	+			+			+		+
RAD51C		+	+	+	+	+	+	+		+
RAD51D			+		+		+	+		+
RINT1			+					+		
STK11	+	+	+	+		+	+	+	+	+
TP53	+	+	+	+	+	+	+	+	+	
XRCC2	+		+				+			+

NOTE. The following is a list of URLs for the providers: Invitae, https://invitae.com/en/test-catalog/; BROCA, http://menu.labmed.uw.edu; myRisk, https://www.myriad.com/products-services/hereditary-cancers/myrisk-hereditary-cancer/; BreastNext, http://www.ambrygen.com/tests/breastnext; Prevention Genetics, https://www.preventiongenetics.com; GeneDX, http://www.genedx.com/test-catalog/disorders/breast-cancer/35589/; Baylor College of Medicine, https://www.bcm.edu/research/medical-genetics-labs/test_detail.cfm?testcode=22404; Emory Genetics, http://geneticslab.emory.edu/tests/MM201; Fulgent Diagnostics, http://fulgentdiagnostics.com/test/breast-ovarian-cancer-ngs-panel/.

Table A2. Full List of Pathogenic Mutations Found in Cases and Controls

Gene	Mutation Type	Nucleotide Change	Protein Change	dbSNP ID	No. of Cases (n = 2,000)	No. of Controls (n = 1,997)
ATM NM_000051.3	Truncating	c.170G>A	p.Trp57*	—	—	1
	Truncating	c.2282_2283del	p.Leu762Valfs*2	—	1	—
	Truncating	c.2413C>T	p.Arg805*	—	1	1
	Truncating	c.2828_2838+14del	p.?	—	1	—
	Truncating	c.3801del	p.Val1268*	—	—	1
	Truncating	c.6048_6051del	p.Ser2017Cysfs*29	—	1	—
	Truncating	c.6403_6404insTT	p.Arg2136*	—	1	—
	Nonsynonymous	c.7271T>G	p.Val2424Gly	rs28904921	1	—
	Truncating	c.8266A>T	p.Lys2756*	—	1	—
	Truncating	c.8307G>A	p.Trp2769*	—	2	—
	Essential splice site	c.8418+1_8418+4del	—	—	—	1
ATR NM_001184.3	Truncating	c.91C>T	p.Gln31*	—	—	1
	Truncating	c.2320_2321insA	p.Ile774Asnfs*3	—	1	—
	Truncating	c.2799_2800del	p.Ile933Metfs*11	—	1	—
	Truncating	c.7273C>T	p.Arg2425*	—	1	—
BARD1 NM_000465.2	Truncating	c.627_628del	p.Lys209Asnfs*4	—	1	—
	Truncating	c.1652C>G	p.Ser551*	—	2	—
	Truncating	c.1752del	p.Met584Ilefs*7	—	—	1
BLM NM_000057.2	Truncating	c.1624del	p.Asp542Thrfs*2	—	1	—
	Truncating	c.1642C>T	p.Gln548*	rs200389141	1	—
	Truncating	c.2084_2085del	p.Ser696Phefs*44	—	—	1
	Truncating	c.2098C>T	p.Gln700*	rs367543028	—	1
	Truncating	c.2517del	p.Asp840Thrfs*3	—	1	—
	Truncating	c.2695C>T	p.Arg899*	—	1	1
	Essential splice site	c.3558+1G>T	—	rs148969222	1	—
BRCA1 NM_007294.3	Truncating	c.66_67insA	p.Glu23Argfs*18	rs80357783	—	1
	Truncating	c.68_69del	p.Glu23Valfs*17	rs386833395	1	1
	Essential splice site	c.594-2A>C	—	rs80358033	—	1
	Truncating	c.1501_1504del	p.Lys501*	rs80357632	—	1
	Truncating	c.4065_4068del	p.Asn1355Lysfs*10	rs80357508	1	—
	Nonsynonymous	c.5207T>C	p.Val1736Ala	rs45553935	2	—
BRCA2 NM_000059.3	Truncating	c.250C>T	p.Gln84*	rs80358515	—	1
	Truncating	c.755_758del	p.Asp252Valfs*24	rs80359659	—	1
	Truncating	c.2539dup	p.Arg847Lysfs*34	—	—	1
	Truncating	c.3103G>T	p.Glu1035*	rs80358556	—	1
	Truncating	c.3744_3747del	p.Ser1248Argfs*10	rs80359403	—	1
	Truncating	c.5213_5216del	p.Thr1738Ilefs*2	rs80359493	1	—
	Truncating	c.5857G>T	p.Glu1953*	rs80358814	—	1
	Truncating	c.6275_6276del	p.Leu2092Profs*7	rs11571658	—	1
	Truncating	c.6402_6406del	p.Asn2135Lysfs*3	rs80359584	—	1
	Truncating	c.7480C>T	p.Arg2494*	rs80358972	—	1
	Nonsynonymous	c.9371A>T	p.Asn3124Ile	rs28897759	1	—
BRIP1 NM_032043.2	Truncating	c.1850T>A	p.Leu617*	—	—	1
	Truncating	c.1871C>A	p.Ser624*	—	2	—
	Truncating	c.2392C>T	p.Arg798*	rs137852986	4	—
	Truncating	c.2990_2993del	p.Thr997Argfs*61	—	1	2
	Truncating	c.2992_2993del	p.Lys998Glufs*3	—	—	1
CDH1 NM_004360.3	Truncating	c.2462_2463insC	p.Thr823Hisfs*6	—	1	—

(continued on following page)

Table A2. Full List of Pathogenic Mutations Found in Cases and Controls (continued)

Gene	Mutation Type	Nucleotide Change	Protein Change	dbSNP ID	No. of Cases (n = 2,000)	No. of Controls (n = 1,997)
CHEK2 NM_007194.3	Nonsynonymous	c.433C>T	p.Arg145Trp	rs137853007	1	—
	Nonsynonymous	c.470T>C	p.Ile157Thr	rs17879961	5	6
	Truncating	c.629_632del	p.Ser210Phefs*6	—	1	—
	Truncating	c.817_818del	p.Glu273Asnfs*16	—	1	—
MRE11A NM_005591.3	Truncating	c.1222_1223insA	p.Thr408Asnfs*49	—	1	—
	Truncating	c.1516G>T	p.Glu506*	—	2	—
	Truncating	c.1897C>T	p.Arg633*	rs137852759	1	—
NF1 NM_000267.3	Truncating	c.3703C>T	p.Gln1235*	—	1	—
	Truncating	c.3826C>T	p.Arg1276*	—	—	1
NBN NM_002485.4	Truncating	c.123del	p.Ser42Alafs*7	—	—	1
	Truncating	c.127C>T	p.Arg43*	rs200287925	1	—
	Truncating	c.657_661del	p.Lys219Asnfs*16	—	1	2
PALB2 NM_024675.3	Truncating	c.172_175del	p.Gln60Argfs*7	—	1	—
	Truncating	c.196C>T	p.Gln66*	rs180177083	2	—
	Truncating	c.522_523del	p.Arg175Thrfs*9	—	1	—
	Truncating	c.577dup	p.Thr193Asnfs*2	—	1	—
	Truncating	c.693dup	p.Gly232Argfs*3	—	1	—
	Truncating	c.758dup	p.Ser254Ilefs*3	—	2	—
	Truncating	c.860dup	p.Ser288Lysfs*15	—	1	—
	Truncating	c.1947dup	p.Glu650Argfs*13	—	1	—
	Truncating	c.1947_1966dup	p.Pro656Glnfs*11	—	1	—
	Truncating	c.2386G>T	p.Gly796*	rs180177112	1	—
	Truncating	c.2391del	p.Gln797Hisfs*54	—	1	—
	Truncating	c.2966_2967insCAACAAGT	p.Glu990Asnfs*3	—	—	1
	Truncating	c.2982dup	p.Ala995Cysfs*16	rs180177127	1	—
	Truncating	c.3113G>A	p.Trp1038*	rs180177132	7	1
	Truncating	c.3116del	p.Asn1039Ilefs*2	rs180177133	1	1
	Truncating	c.3256C>T	p.Arg1086*	—	1	—
	Truncating	c.3362del	p.Gly1121Valfs*3	—	1	1
	Truncating	c.3507_3508del	p.His1170Phefs*19	—	1	—
	Truncating	c.3549C>G	p.Tyr1183*	rs118203998	1	—
PTEN NM_000314.4	Truncating	c.388C>T	p.Arg130*	rs121909224	1	—
RAD50 NM_005732.3	Truncating	c.541_542delinsA	p.Ser181Lysfs*9	—	—	1
	Truncating	c.1017dup	p.Asn340Glnfs*10	—	—	1
	Truncating	c.1188del	p.His397Thrfs*4	—	—	1
	Truncating	c.1958C>A	p.Ser653*	—	—	1
	Truncating	c.2165dup	p.Glu723Glyfs*5	rs397507178	—	1
	Truncating	c.2983_2986del	p.Glu995Argfs*2	—	—	1
	Truncating	c.3029_3032del	p.Thr1010Argfs*14	—	1	—
	Truncating	c.3209del	p.Asn1070Ilefs*6	—	1	—
TP53 NM_000546.5	Nonsynonymous	c.473G>A	p.Arg158His	—	1	—
	Nonsynonymous	c.524G>A	p.Arg175His	rs28934578	1	—
	Truncating	c.717_718del	p.Asn239Lysfs*24	—	1	—
	Nonsynonymous	c.733G>A	p.Gly245Ser	rs28934575	1	—
	Nonsynonymous	c.746G>A	p.Arg249Lys	—	1	—
XRCC2 NM_005431.1	Truncating	c.651_652del	p.Cys217*	—	1	—
	Truncating	c.683delG	p.Cys228Leufs*8	—	1	—