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EPIDEMIOLOGY

Allelic modification of breast cancer risk in women with an *NBN* mutation

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Abstract

Background *NBN* 657del5 founder mutation predisposes to breast and prostate cancer. Recently, it has been reported that the pathogenicity of this mutation with regard to prostate cancer risk is modified by a missense variant of the same gene (E185Q).

Methods To evaluate the interaction of the 657del5 and E185Q founder alleles of *NBN* on breast cancer risk in Poland, 4964 women with breast cancer and 6152 controls were genotyped for these two recurrent variants of *NBN* (657del5 truncating variant and E185Q missense variant).

Results The *NBN* 657del5 mutation was detected in 57 of 4964 unselected cases and in 35 of 6152 controls (OR = 2.0, $p = 0.001$). The E185Q GG genotype was detected in 2167 of 4964 unselected cases and in 2617 of 6152 controls (OR = 1.04, $p = 0.3$). In carriers of the 657del5 deletion, the elevated cancer risk was restricted to women with the GG genotype of the E185Q variant (OR = 3.6, 95% CI 1.9–6.6; $p < 0.0001$). Among women with other E185Q genotypes, the OR associated with 657del5 was 1.0 (95% CI 0.5–1.8; $p = 0.9$). The interaction between the two alleles was statistically significant (homogeneity $p = 0.003$).

Conclusion In Poland, the pathogenicity of the *NBN* 657del5 mutation is restricted to women with a homozygous GG genotype of missense variant of the same gene (E185Q). This is the first clear example whereby a moderate penetrance breast cancer gene is impacted by a genetic modifier.

Keywords *NBN* · *NBS1* · Mutation · Breast cancer

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Introduction

There are perhaps a dozen high-penetrance and moderate-penetrance breast cancer genes identified to date, which together account for about ten percent of all breast cancer cases [1]. The risk of cancer differs by gene and also may differ for various alleles of the same gene. It has been proposed that genetic background may affect risk-that is, two individuals with the same mutation may face different cancer risks based on allelic differences in the same gene or in other genes [2–4]. There is interest in identifying genetic modifiers of risk for carriers of cancer mutations with the hope that these may help explain incomplete penetrance and that they can be used for determining risk in individual women. To this effect, in 2013 a genome-wide study sought to identify clinically important genetic modifiers of *BRCA1* mutations, but none were found [5]. The example of *BRCA1*, however, may not be indicative of the situation for other cancer genes.

In Poland, there are 20 founder alleles in six cancer genes, which account for 82% of all mutations and 52% of familial cases [6]. A single allele of the *NBN* (*NBS1*) gene (657del5) is a founder mutation in Poland, and accounts for about 1% of all breast cancer cases in the country [7, 8]. To date, the genetic epidemiology of *NBN* is based mostly on studies of Polish cases and these results may not be generalizable to other genetic groups. In particular, the pathogenicity of other *NBN* variants (with regard to cancer risk) has not been established [9, 10]. The product of the *NBN* gene is part of the genome surveillance complex responsible for DNA damage repair [11]. Homozygous carriers of *NBN* mutations are diagnosed with the Nijmegen Breakage Syndrome (NBS), which features immunodeficiency, chromosomal instability, microcephaly as well as a predisposition to various cancers. A 5-bp deletion in exon 6 of the *NBN* gene (657del5) in homozygous state is present in the majority of NBS patients [12]. Heterozygous carriers of the 657del5 truncating mutation exhibit increased susceptibility to breast and prostate cancer [3, 6–10].

There is a common missense variant in the *NBN* gene, E185Q, which on its own does not predispose to prostate cancer, but modifies the risk of prostate cancer in carriers of the classical founder allele 657del5 [3]. That is, among men with the GG genotype, the OR associated with 657del5 was 4.4 (95% CI 2.4–8.0), and among men with other E185Q genotypes, the OR associated with 657del5 for prostate cancer risk was 1.4 (95% CI 0.8–2.4) and the interaction was significant (homogeneity p=0.006). A modifying effect of E185Q was seen on prostate cancer survival as well [3]. Given that NBN is a multi-site cancer gene, it is important to know if the allelic interaction is specific to prostate cancer or represents a more general phenomenon. In an earlier study, based on 2012 cases and 4000 controls, we estimated that the 657del5 allele confers approximately twofold increased risk for breast cancer in Poland [7]. In the present study, we revisit the patient group and ask if the risk of breast cancer in *NBN* 657del5 carriers is modified by the E185Q variant.

Materials and methods

Patients

We studied women with unselected breast cancer who were diagnosed between 1999 and 2015 in one of 14 centers in Poland. Women with newly diagnosed breast cancer were invited to participate in this study. Women enrolled in the current study were unselected for family history, clinical characteristics, and treatment. Study subjects were asked to participate at the time of diagnosis or during an outpatient visit to an oncology clinic. All patients provided a blood sample within 6 months of diagnosis. The mean age of diagnosis was 49 years (range 22–92 years). A family history was taken either by the construction of a family tree or the completion of a standardized questionnaire. 744 women reported at least one first- or second-degree relative with breast cancer (familial cases). The control group included 6152 cancer-free adults from Poland. The control group consisted of 3157 cancer-free men age 23–90 years (mean age, 62.2 years) and 2995 cancer-free women age 18–94 years (mean age, 54.0 years) [3]. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland.

In a secondary analysis, we identified 245 men and women who had attended the outpatient cancer genetics clinic of the Pomeranian Medical University and who had been found to carry the 657del5 *NBN* mutation. (The allele is present in approximately one in 160 Polish individuals.) Of these, 54 were women with breast cancer, 54 had another type of cancer and 137 had not been diagnosed with cancer. All 245 subjects were genotyped for the presence of the E185Q alleles (CC, CG, GG). None of the 245 subjects were included in the first part of the study, described above.

Genotyping

DNA was isolated from 5 to 10 mL of peripheral blood. *NBN* mutations were genotyped using TaqMan assay (Thermo Fisher Scientific) using LightCycler® Real-Time PCR 480 System (Roche Life Science).

Statistical analysis

The frequencies of all alleles in cases and controls were compared. Odds ratios (OR) were generated from two-by-two tables and statistical significance was assessed with the Fisher exact test or the Chi-squared test where appropriate. The ORs were used as estimates of relative risk. The Breslow-Day test was used for testing the homogeneity of odds ratios.

Results

The *NBN* 657del5 mutation was detected in 57 of 4964 unselected breast cancer cases (1.4%) compared to 35 of 6152 (0.6%) controls (OR = 2.0; p=0.001). The *NBN* E185Q GG genotype was detected in 2167 of 4964 unselected cases (43.6%) compared to 2617 of 6152 (42.5%) controls (OR = 1.0; p=0.3) (Table 1). Among women with the E185Q GG genotype the odds ratio associated with the 657del5 mutation was 3.6 (95% CI 1.9 to 6.6). Among women with

Table 1 Associations of various *NBN* alleles with breast cancer risk

NBN variant	Unselected breast cancer cases			Familial breast cancer cases			Controls*
	No/total (%)	OR (95% CI)	p value	No/total (%)	OR (95% CI)	p value	No/total (%)
657del5	57/4964 (1.1%)	2.0 (1.3–3.1)	0.001	11/744 (1.5%)	2.6 (1.3–5.2)	0.008	35/6152 (0.6%)
E185Q (GG genotype)	2167/4964 (43.6%)	1.0 (1.0–1.3)	0.3	322/744 (43.3%)	1.0 (0.9–1.2)	0.7	2617/6152 (42.5%)
E185Q (GC genotype)	2213/4964 (44.6%)	1.0 (0.9–1.0)	0.3	330/744 (44.3%)	1.0 (0.8–1.1)	0.6	2798/6152 (45.5%)
E185Q (CC genotype)	584/4964 (11.8%)	1.0 (0.9–1.0)	0.8	92/744 (12.4%)	1.0 (0.8–1.3)	0.8	737/6152 (12.0%)

*p values are calculated with respect to the variant frequency in controls

Table 2 Effect of *NBN* 657del5 on breast cancer risk by genotype of the E185Q missense variant

Variants present	Unselected breast cancer cases			Controls no.
	No.	OR (95% Cl)	p value	
NBN 657del5-negative and GG genotype	2126			2603
NBN 657del5-positive and GG genotype	41	3.6 (1.9–6.6)	< 0.0001	14
NBN 657del5-negative and GC/CC genotype	2781			3514
NBN 657del5-positive and GC/CC genotype	16	1.0 (0.50–1.8)	0.9	21

Table 3 Clinical characteristics of breast cancers in carriers of variant alleles in *NBN*

	No 657del5 mutation (n = 4902)	NBS1 657del5 mutation (n = 57)	NBS1 657del5 and E185Q GG genotype (n = 41)	NBS1 657del5 positive and E185Q non-GG genotype (n = 16)
Mean age of diagnosis (years)	48.9	48.0	48.9	45.8
Family history of breast cancer	733/4902 (15%)	11/57 (19%)	8/41 (20%)	3/16 (19%)
Receptor status				
ER positive	2518/3869 (65%)	34/50 (68%)	22/35 (63%)	12/15 (80%)
PR positive	2536/3681 (69%)	32/50 (64%)	22/35 (63%)	10/15 (67%)
HER2 positive	571/3013 (19%)	7/37 (19%)	5/27 (19%)	2/10 (20%)
Tumor size (cm)				
< 1	356/3827 (9%)	7/49 (14%)	5/34 (15%)	2/15 (13%)
1–1.9	1513/3827 (39%)	19/49 (39%)	11/34 (32%)	8/15 (53%)
2–4.9	1723/3827 (45%)	23/49 (47%)	18/34 (53%)	5/15 (33%)
≥ 5	186/3827 (5%)	–	–	–
Lymph node status				
Lymph node positive	1766/3890 (45%)	21/47 (45%)	12/34 (35%)	9/13 (69%)

other E185Q genotypes (CG or CC) the odds ratio associated with the 657del5 mutation was 1.0 (95% CI 0.5 to 1.8) (Table 2). The statistical test to reject homogeneity of the odds ratio was highly significant (p=0.003). The breast cancers in the 657del5 mutation carriers and non-carriers were similar (Table 3).

We identified an additional 54 women with breast cancer and *NBN* 657del5 mutation and 137 cancer-free carriers of *NBN* 657del5 from our outpatient clinical records. There was no overlap between these cases and the 92 *NBN* 657del5 mutation positive cases described above. We found that among carriers of 657del5 allele, the GG genotype of E185Q was present in 40 of the 54 (74%) breast cancer cases compared to 66 of the 137 (48%) individuals who had no cancer diagnosed (OR = 3.1; 95% CI 1.5–6.2; p=0.001).

Discussion

In 2003, we first described the association between a small deletion of *NBN (NBS1)* and breast cancer risk [13]. In 2013, we estimated the contribution of *NBN* 657del5 mutation to unselected breast cancer cases in Poland to be 1.2% of all cases [8]. The *NBN* 657del5 founder mutation predisposes to prostate cancer as well (and probably other *NBN* protein-truncating mutations) [14–18]. In 2018, we reported that among men with *NBN* 657del5 deletion, only those with the GG E185Q genotype of *NBN* were predisposed to prostate cancer [3]. That study represented the first example of a hereditary cancer syndrome where the penetrance of a pathogenic allele is conditional on the genotype an allele elsewhere in the same gene. In the current study, we hereby confirm that the genetic interaction holds true for breast cancer risk as well, that is, the breast cancer risk among 657del5 carriers is restricted to women with two copies of the E185Q G allele. [The odds ratio for carriers of 657del5 and the GG genotype of E185Q was 3.6, compared to odds ratio of 1.0 for 657del5 carriers with other E185Q genotypes (GC or CC).] The p value for heterogeneity was highly significant and the interaction between 657del5 and E185Q with regard to breast cancer risk was preset in two independent data sets of breast cancer patients and controls.

To our knowledge, this is the first clear example of a two-allele model for hereditary breast cancer. Couch and colleagues conducted a GWAS study of 5920 *BRCA1* carriers (affected and unaffected) and failed to find a strong risk modifier [5]. The only locus with a significant association for *BRCA1* carriers was rs2290854 on chromosome 1q32, but the associated hazard ratio was only 1.14. Given such a small effect size, the difference in penetrance of carriers with and without the second risk allele is expected to be negligible and without clinical implications. In 2019, there is no test for genetic risk modifiers that is in clinical use. We propose that the *NBN* association is the first genetic modifier that is sufficiently well-documented to be used to base clinical decisions. Specifically, we will counsel Polish carriers of *NBN* 657del5 mutation based on the joint genotypes and will restrict high risk surveillance protocols to those with the GG genotype of E185Q. *NBN* is currently included on several commercial cancer gene panels. We recommend that caution be employed when counseling women with other *NBN* mutations. We recommend that other databases be studied for evidence of pathogenicity of other variants prior to incorporating the information into clinical care.

In conclusion, we describe a new model for inherited breast cancer susceptibility wherein the penetrance of a dominant cancer susceptibility gene *(NBN)* is modified by another allele of the same gene (in *trans* or in *cis*). The G allele of E185Q is in complete linkage disequilibrium with the 657del5 mutation, and we found that all chromosomes with the 657del5 mutation have the G allele of E185Q. It was observed that 72% of unselected breast cancer cases with the 657del5 deletion have the G allele on the other (non-mutant) chromosome (compared to only 40% among cancer-free controls). This data suggests that among the deletion carriers it is necessary that the normal copy of the NBN protein carries a glutamic acid residue at position 185 (that corresponds to allele G of E185Q) to confer elevated susceptibility to breast cancer (and prostate cancer). This phenomenon may help explain to some degree why different risks of cancer have been reported for mutations in the same gene in different countries. It will be important to study this paradigm in other cancer genes as well. We recommend exercising caution when it comes to generalizing findings from one cancer mutation to another.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin.

Informed consent Informed consent was obtained from all individual participants included in the study.

Appendix

Other members of Polish Hereditary Breast Cancer Consortium are: M. Bębenek, D. Godlewski, S. Gozdecka-Grodecka, S. Goździ, O. Haus, H. Janiszewska, M. Jasiówka, E. Kilar, R. Kordek, B. Kozak-Klonowska, G. Książkiewicz, A. Mackiewicz, E. Marczak, J. Mituś, Z. Morawiec, S. Niepsuj, R. Sibilski, M. Siołek, J. Sir, D. Surdyka, A. Synowiec, C. Szczylik, R. Uciński, B. Waśko, R. Wiśniowski, T. Byrski, B. Górski, M. Lener, J. Tomiczek-Szwiec, J. Jarkiewicz-Tretyn, M. Cechowska, P. Domagała.

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