

Germline *NBS1* Mutations in Families with Aggregation of Breast and/or Ovarian Cancer from North-East Poland

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Abstract. *Background:* *NBS1* gene, which product participates in DNA repair, has been postulated to be a susceptibility factor for a number of types of cancer, including breast cancer. The carrier frequency of the 657del5 and I171V *NBS1* gene mutations among Polish patients with familial breast and/or ovarian cancer was compared with that of randomly selected newborns. *Patients and Methods:* Using allele-specific amplification-polymerase chain reaction (ASA-PCR) and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) techniques, blood samples were analysed from 250 patients with breast or/and ovarian cancer and a total number of 4,000 for 657del5 mutation and 1,300 for I171V mutation controls. *Results:* Although an increased frequency of both mutations in cancer cases – 0.8% of 657del5 and 2.4% of I171V, compared to controls – 0.52% and 1.38%, respectively, was found, the differences were not statistically significant. *Conclusion:* Our results indicate that *NBS1* mutations do not contribute significantly to breast or ovarian cancer development.

Germline mutations in the two major susceptibility genes, *BRCA1* and *BRCA2*, are associated with high penetrance autosomal dominant mode of inheritance of 5-10% of all breast and ovarian cancer patients (1). An increased risk of breast cancer has also been reported for heterozygous mutation carriers of the ataxia telangiectasia-mutated gene (*ATM*) (2). Familial aggregation of breast cancer may also occur due to an accumulation of multiple low penetrance genes or combinations of recessive alleles (3).

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disorder and the most frequent mutation among NBS patients is a homozygous alteration in the *NBS1* gene,

657del5. NBS homozygotes are characterised by the triad of spontaneous chromosomal instability, immunodeficiency and a significant predisposition to cancer. Malignant disease, particularly lymphoma and leukaemia, have been observed in over a third of NBS patients under the age of 21 (4).

The majority of patients with NBS carry the homozygous mutation 657del5 in the *NBS1* gene, resulting in truncated and rapidly degraded protein (5, 6). Since heterozygous carriers of this mutation have been found with high frequency (1/177) in the Slavic population, this deletion is thought to be a founder mutation. It is estimated that approximately 0.5% of the Eastern European population harbour the heterozygous 657del5 mutation in *NBS1* (6).

It has been suggested that, similarly to the *ATM* gene, the heterozygous carriers of founder Slavic mutations may be at increased risk of developing breast cancer. To date, mutations in *NBS1* have been found in children with lymphoblastic leukaemia and non-Hodgkin's lymphoma (7-10). It has been suggested that carriers of the 657del5 mutation have a higher risk of other malignant tumours, especially melanoma and ovarian, prostate and breast cancer (9, 11-14). Therefore it was hypothesised that *NBS1* acts as a tumour suppressor, but so far few results have supported this hypothesis.

An increased number of carrier frequency of another germinal mutation of the *NBS1* gene, I171V, in a group of 47 juvenile acute lymphoblastic leukaemia (ALL) patients was first reported by Varon *et al.* (8). This point mutation occurs in the breast cancer carboxyl-terminal (BRCT) domain that is involved in protein – protein interactions. A recent study has demonstrated a high frequency of heterozygous 511A>G transition, leading to a substitution I171V in patients with childhood ALL (15). A homozygous I171V mutation carrier was also detected in a group of 62 patients with aplastic anaemia and other haematological patients (16).

In this report, we analysed the carrier frequency of the 657del5 and I171V *NBS1* gene mutations in a group of Polish patients with familial breast and/or ovarian cancer and geographically matched newborns.

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Patients and methods

Patients. Blood samples were analysed from 250 patients affected with breast and/or ovarian cancer (181 patients with breast cancer, 28 patients with ovarian cancer and 41 patients with both malignancies). Families were accrued essentially as "research" families ascertained at the Regional Oncological Outpatient Clinic in Gdansk, Poland, in the period 1999-2005. Informed consent was obtained from all the patients and the study was approved by the Medical Review Board of Gdansk Medical University. Genetic testing was offered to any family whose members met either or both of the following criteria: i) three first- or second-degree relatives (from one parental side of the family) with breast or ovarian cancer diagnosed at any age; ii) two affected family members with breast or ovarian cancer, with at least one of the cancers diagnosed before the age of 50. The average age at diagnosis of breast cancer was 48.3 (27-69) years and ovarian cancer 45.7 (29-63) years. Of all patients, 16% were positive for *BRCA1* mutations.

Controls. A total of 4,000 and 1,300 randomly selected anonymous blood samples on Guthrie cards drawn from newborns from the screening programmes of North-East Poland, were analysed for 657del5 and I171V mutations, respectively.

DNA isolation. DNA from all patients was extracted from whole blood. Red blood cells were lysed by Red Blood Cell lysing buffer (RBC, St. Louis, USA). DNA was extracted using the standard phenol-chloroform protocol (17). Pieces approximately 2 mm in diameter were cut from each Guthrie card and used in PCR reaction.

Mutation detection. The 657del5 mutation was analysed by allele-specific amplification polymerase chain reaction (ASA-PCR) using the following oligonucleotide primers: NBS1Ex6f: 5'TGG AAA TTA TGC CTT TTG AGT GT3'; NBS1Ex6r: 5'CAT GAT CAC TGG GCA GGT CT3'; NBS1Ex6del5: 5'TCA GGA CGG CAG GAA AGA AAT3'.

The I171V missense mutation was identified by restriction fragment length polymorphism PCR (RFLP-PCR) using *BseNI* enzyme (Fermentas) and the following primers: NBS1Ex5F: TTA TGG ATG TAA ACA GCC TC and NBS1Ex5R: TAC CGA ACT ATA ACA CAG CA. The *BseNI* enzyme recognises the altered sequence (A>G), resulting in the digestion of mutated PCR product. Both positive and negative controls were used to determine the presence or absence of the mutation.

PCR products were separated on 2% agarose stained with ethidium bromide and visualised in UV light. All positive cases were confirmed using sequencing. For sequencing reaction, exon 6 of the *NBS1* gene was amplified by PCR using the following primers: NBS1ex6F 5'TTA TTT TTA CAG TTT TTA CCC ACC TC3' and NBS1ex6R 5'ACA TAA TTA CCT GTT TGG CAT TCA3'. PCR products were purified and sequenced using a DNA Kit with Big Dye Terminator (Applied Biosystems, Foster City, USA). Sequenced products were analysed in ABI PRISM 310 DNA Sequencer (Applied Biosystems, Foster City, USA).

Statistical analysis. The frequency of the mutation in the study group and controls was compared using Chi-square and Fisher exact tests.

Table I. Frequencies of heterozygous mutation carriers of the *NBS1* gene in a group of familial breast and/or ovarian cancer cases and controls.

Mutation	Frequency in patients	Frequency in controls
657del5	2/250 0.8%	21/4000 0.52%
I171V	6/250 2.4%	18/1300 1.38%

Results

In a group of 250 patients, eight patients (3.2%) harboured the heterozygous mutation in *NBS1*. The 657del5 allele was detected in two cases (0.8 %) (Table I). Both patients were affected with breast cancer and were negative for *BRCA1*. History of both patients revealed two cases of breast cancer in each family and no ovarian cancer.

The I171V allele was found in six cases (2.4%). One of six carriers of this mutation was affected with both breast and ovarian cancer, four carriers with breast cancer and one with bilateral breast cancer. Family history of one patient revealed, besides ovarian cancer, two incidents of prostate cancer, but its late onset suggests sporadic rather than hereditary origin. In one family there were three cases of breast cancer and this was the most affected family. All patients carrying the I171V mutation were negative for *BRCA1* mutations.

Among 4000 controls, *NBS1* 657del5 heterozygous mutation was present in 21 (0.52%) individuals. The missense I171V mutation was found in 18 out of 1300 samples, which gives a frequency of 1.38%. Carrier frequencies of both mutations among patients and controls were not significantly different ($p>0.05$).

Discussion

Our analysis of 4000 control blood samples on Guthrie cards revealed 21 heterozygous 675del5 mutations giving an overall frequency of 1/190. This result remains in concordance with a previous study in which the mean prevalence of 657del5 mutation in the three Slav populations (Czech Republic, Ukraine and Poland) was found to be 1/177 (6). However, in Poland, a differential regional distribution of heterozygous 657del5 mutation is observed. The frequency observed in our research is lower than in the studies of the Wielkopolska region (1/131) (18) and Mazovian voivodeship (1/162) (14), but higher than that obtained from North-West Poland. In that study, the frequency of Slavic mutation in the mixed control group (new-borns and adults) was 1/222 (19).

Some recent studies suggest a correlation between carrying *NBS1* mutations and breast/ovarian cancer. Analysis of a large group of unselected cases revealed an approximately 3-

fold increase in 657del5 mutation frequency (14). All *NBS1* mutation carriers detected in the aforementioned report had no family history of disease. Statistically significant difference was also found between a group of 80 families with at least 3 first-degree relatives affected with breast cancer and 530 general population individuals (20). These findings remain clearly in contrast with other research. Results obtained in a cohort of Russian patients proved a significantly higher cancer risk only in a group of bilateral breast cancer patients (21). Comprehensive studies on a consecutive group of breast cancer patients did not confirm the hypothesis that the 657del5 mutation significantly contributes to breast cancer risk in the German population (22).

A Polish study in a large group of 2012 unselected breast cancer cases also revealed no significant association between 657del5 mutation and breast cancer (20). Our study was performed on patients with familial aggregation of breast/ovarian cancer. Although increased frequency of 657del5 deletion was detected, statistical significance could not be proven.

In our study, no mutation carrier of the 657del5 mutation was found among 69 patients with ovarian cancer. This result is similar to the findings of a recent study, where the analysis of 108 patients with ovary and oviduct tumour revealed no carrier of this deletion (9).

In the present study, we also screened all patients for the *NBS1* gene I171V missense mutation to investigate whether it plays a role in breast or ovarian cancer development. Interestingly, in a large series of 1300 controls we demonstrated a higher frequency of this mutation (1/72) than in a recent Polish study (1/500) (15); this was only slightly higher in comparison to the 1/82 frequency reported in a Japanese study (16). This might be due to geographical differences in the distribution of the mutated allele.

So far, this missense mutation has only been described among patients with childhood acute lymphoblastic leukaemia (ALL), aplastic anaemia (AA) or non-Hodgkin's lymphoma (NHL) and this mutation was of germline or/and somatic origin (8, 15, 16). In our study, in a group of 250 patients with breast or/and ovarian cancer, we observed increased carrier frequency in comparison with the control group but the difference was not significant. All previous analyses were based on the assumption that the I171V mutation disrupts protein – protein interaction domains. However, functional assays, necessary to confirm the pathogenicity of this alteration, have never been performed.

In our study, all patients who carry the mutation in the *NBS1* gene were negative for *BRCA1* mutation. Antoniou *et al.* (3) have suggested that susceptibility to breast cancer in non-carriers of *BRCA1* mutations may be mainly attributed to a 'polygenic' model, with a large number of susceptibility alleles, each conferring small risk but together producing a synergistic effect on disease risk. The pattern of breast cancer

risk among *NBS1* mutation carriers would appear to be consistent with such a model, since the *NBS1* product is an integral component of the hMRE11/hRAD50/*NBS1* nuclease complex. These results indicate that *NBS1* gene mutations may play a role in breast/ovarian cancer development in association with mutations in other low penetrance genes and suggests further study of the genes encoding for proteins involved in the same DNA repair pathway.

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