

The NOD2 3020insC Mutation in Women with Breast Cancer from the Bydgoszcz Region in Poland. First Results

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Key words: breast cancer, *NOD2* gene mutation

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Submitted: 20 December 2005

Accepted: 10 January 2006

Abstract

The frameshift *NOD2* gene mutation 3020insC is predominantly associated with Crohn's disease, but predisposes to many types of common cancers as well. We studied the frequency of this mutant *NOD2* allele in 148 breast cancer women from the Bydgoszcz region in Poland. The *NOD2* mutation was present in 8.8% of the patients. The mean age at breast cancer diagnosis of the mutation carriers was 43 years. We did not find any mutation in patients diagnosed with breast cancer after the age of 50 years. There was no association of the *NOD2* mutation with a strong family history of breast cancer. On the contrary, the mutation frequency (11.4%) was two times higher in women from families with a single case of breast cancer and with aggregation of other common types of cancer, especially digestive tract cancers. Low risk of breast cancer in the mutation carriers seems to be confirmed by finding the 3020insC mutation in three healthy parents of probands aged 73, 74 and 83 years, from three separate families.

Introduction

The *NOD2* gene has been identified and mapped to chromosome 16q12 by Hugot and coworkers [1]. It consists of 12 exons and its product, a cytosolic protein, consists of 1,040 amino acids. The *NOD2* protein contains three functional domains: an amino-terminal effector-binding domain (EBD) consisting of two caspase-recruitment domains (CARD), a centrally located nucleotide-binding oligomerization domain (NOD), and a carboxy-terminal ligand-recognition domain (LRD) consisting of ten carboxy-terminal leucine-rich repeats (LRRs) [2].

The *NOD2* protein is involved in the inflammatory response and in the activation of nuclear factor- κ B (NF- κ B)

through interaction with the CARD domains after stimulation of monocytes by bacterial products [2, 3]. Activation of NF- κ B is stronger when mutation 3020insC occurs in the *NOD2* gene [2, 4]. A cytosine insertion in exon 11 causes a frameshift mutation and, as a consequence, amino acid substitution at position 1007 (Leu1007fsinsC) of the *NOD2* protein. This provokes premature termination of protein synthesis, with a loss of the last 33 amino acids [3].

The mutation predisposes predominantly to Crohn's disease, a common chronic inflammatory bowel disease that is known to favour colorectal cancer development [3-5]. It was recently found that the 3020insC mutation of *NOD2* also predisposes to many types of common cancers, e.g. breast cancer [6-9]. The mu-

tant allele is more frequently found in women with early-onset breast cancer and in women with ductal breast cancer with an *in situ* component [8].

In this report we present our preliminary data of the NOD2 3020insC allele study in women from the Bydgoszcz region (north-central Poland), diagnosed with breast cancer.

Patients and methods

A total number of 148 breast cancer patients were included in the study between 2003 and 2005 from the Oncology Center and Oncogenetic Counseling Clinic of the University Hospital in Bydgoszcz. The patients were unselected for age, histopathological type of breast cancer and family history. The age of breast cancer onset was in the range from 23 to 79 years. 112 women were diagnosed under the age of 50, and 36 women ≥ 50 .

Among the studied patients, 69 had a family breast cancer history with at least two breast cancers in first- or second-degree relatives and 79 had a single relative with breast cancer. In 104 out of 148 (70.3%) families, an aggregation of cancers of colon, pancreas, stomach, larynx, lung, ovary, prostate, kidney, and melanoma, leukaemia or sarcoma occurred, in addition to breast cancer. In six families with 3020insC, mutation analysis of healthy first- and second-degree relatives of the probands was performed (15 persons: 6 men and 9 women). Family members with other types of cancer could not be screened for mutations because all were deceased at the time of this study.

Medical records confirmed the diagnosis of cancer and the clinical history of all patients and their families. Informed consent was obtained from all investigated persons.

Genomic DNA was extracted from peripheral blood leukocytes by the standard method with proteinase K. The

Table 1. The NOD2 3020insC mutation detected in breast cancer families from the Bydgoszcz region of Poland

Family	Investigated family members				Family, including proband	
	Age of onset (years)		Age of mutation diagnosis (years)		Type and (number) of cancers	
	DNA	Breast	Healthy females	Healthy male	Breast	Others
1.	3362 ^a	49	–	–	2	prostate (1), digestive tract (1), sarcoma (1)
	2258	–	83	–		
2.	3432 ^a	44	–	–	1	digestive tract (1), uterus (2)
	2279	–	73	–		
3.	3307 ^a	48	–	–	1	–
	2265	–	23	–		
4.	3355 ^a	49	–	–	4	liver (1), myeloma (1)
	2313	–	–	74		
5.	3371 ^a	41	–	–	1	lung (1)
	2285	–	39	–		
6.	3390 ^a	49	–	–	3	stomach (1), lung (1), pancreas (2), sarcoma (1)
7.	3333 ^a	43	–	–	1	larynx (1), pancreas (1)
8.	3465 ^a	35	–	–	1	kidney (1), colon (1),
9.	3452 ^a	37	–	–	1	colon (1), lung (1)
10.	3309 ^a	38	–	–	1	cervix uteri (1), liver (1)
11.	3348 ^a	38	–	–	1	–
12.	3312 ^a	47	–	–	2	–
13.	3323 ^a	42	–	–	1	lung (1), ? (1)

^a proband

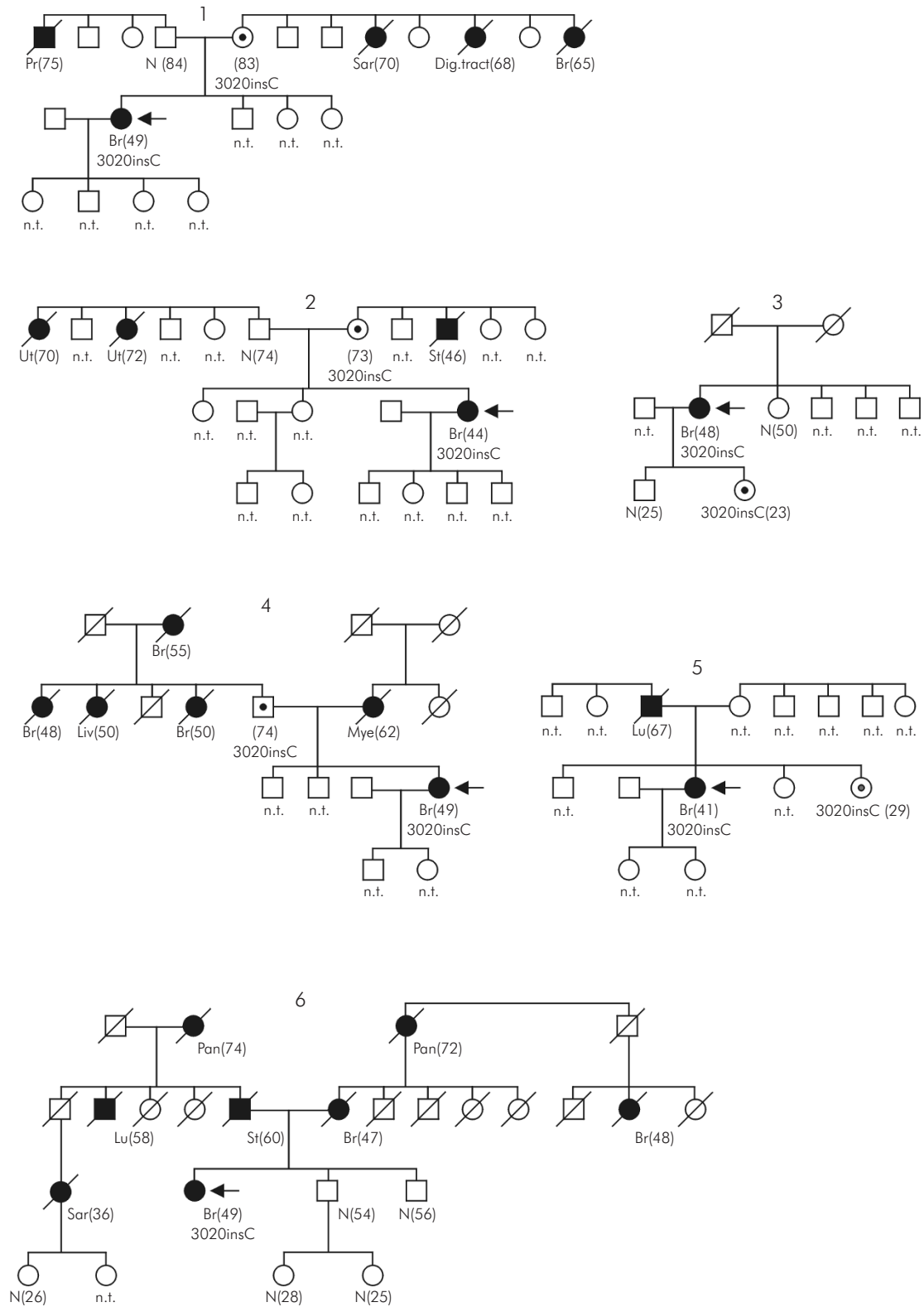


Fig. 1. Pedigrees of families with the *NOD2* 3020insC mutation: families 1-6. Black symbols = persons affected with cancer; white symbols with a black dot inside = healthy mutation carriers. Age of cancer onset or age of *NOD2* mutation diagnosis in healthy persons is given in brackets. Br = breast cancer; Dig.tract = digestive tract cancer; Liv = liver cancer; Lu = lung cancer, Mye = myeloma; Pan = pancreatic cancer; Pr = prostate cancer; Sar = sarcoma; St = stomach cancer; Ut = uterus cancer. Arrows indicate Probands. 3020insC = mutation carriers; N = analysed persons who are not carriers of the mutation; n.t. = not tested

molecular investigations of 3020insC were carried out by an allele-specific PCR assay using the kit produced by Molecular Laboratory, Pomeranian Medical University of Szczecin, Poland (patent no. P-364412, Poland) [8].

The PCR product was digested by Apal restriction enzyme. After electrophoretic separation two fragments, 319bp-wild allele and 214bp-mutant allele, were observed.

Results

The 3020insC *NOD2* gene mutation was detected in 13 of the 148 breast cancer patients (8.8%). The mean age of the mutation carriers at breast cancer diagnosis was 43 years (range 35-49 years). The mutation was found in 11.6% (13/112) of women diagnosed with breast cancer before the age of 50 and in none diagnosed at or after the age of 50. It was detected in 9 of 79 families (11.4%) without breast cancer history and in 4 of 69 families (5.8%) with at least two cases of breast cancer. In 10 out of 13 (85%) families with the mutation, an aggregation of other common types of cancer occurred, besides breast cancer (Table 1). In 6 families, molecular investigations of first- and second-degree relatives of probands were performed. All tested relatives were healthy at the time of mutation analysis (Figure 1. Families 1-6). In Family 1, two breast cancers (mean age of onset 57 years), one prostate cancer, one digestive tract cancer and one sarcoma had occurred. The mutation was detected in the proband, who was diagnosed with breast cancer at the age of 49 and in her 83-year-old healthy mother (Fig. 1.1). In Family 2, one breast cancer in the proband (age of onset 44 years), one digestive tract cancer and two uterine cancers occurred. Carrying of the mutation was detected in the proband and in her 73-year-old healthy mother (Fig. 1.2). In Family 3, only one cancer – in the proband (age of onset 48 years) – was diagnosed. The mutation was identified in the proband and in her 23-year-old healthy daughter (Fig. 1.3). In Family 4, four breast cancers (mean age of onset 50.5 years), one liver cancer, and one myeloma occurred. The mutation was found in the proband, diagnosed with breast cancer at the age of 49, and in her 74-year-old healthy father (Fig. 1.4). In Family 5, one breast cancer in the proband (age of onset 41 years) and one lung cancer occurred. The mutation was detected in the proband and in her 39-year-old healthy sister (Fig. 1.5). In Family 6, three breast cancers (mean age of onset 48 years), one stomach cancer, one lung cancer, two pancreatic cancers and one sarcoma occurred. The mutation could only be demonstrated in the proband (age of onset 49 years) (Fig. 1.6).

Discussion

The *NOD2* 3020insC allele is relatively common (7.3%) in the Polish population [7]. Previously it has been reported in 8.0% of 462 breast cancer patients from Szczecin [8]. In the present study, among 148 women with breast cancer from the Bydgoszcz region, similarly, a frequency of 8.8% was observed. The mutation was found with higher frequency in the group of women diagnosed with breast cancer before the age of 50 (13.2% in Szczecin and 11.6% in the Bydgoszcz region) [8, present paper]. In the study of the Szczecin region, the authors found a modest, not statistically significant, association of the *NOD2* 3020insC mutation with family breast cancer history [8]. In our study no such association could be demonstrated. On the contrary, the frequency of the mutant allele (11.4%) was two times higher in women from families with a single case of breast cancer than in women from families with a stronger breast cancer family history. Interestingly, in seven out of nine 3020insC families without breast cancer history, an aggregation of other common types of cancer occurred. In these families the most frequent types of cancers were digestive tract cancers such as gastric, pancreatic and colon cancer.

The population risk of breast cancer before the age of 50, associated with the *NOD2* mutation, is approximately 1%. It increases only two times in this age group of 3020insC carriers, but does not increase in older age groups [8]. This observation of low risk of breast cancer in mutation carriers [8] is supported by our findings. Three patients with 3020insC, diagnosed with breast cancer at the age of 44, 49 and 49, had one healthy mutation-carrying parent (two mothers and one father) at the age of 73, 74 and 83, respectively. Our study suggests that the *NOD2* 3020insC mutation might predominantly increase the risk of developing digestive tract cancer rather than breast cancer, but studies on larger groups of patients are needed to further clarify the tumour spectrum and cancer risk figures associated with this mutation.

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