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*Brief communication*

# The 3020insC allele of NOD2 predisposes to early-onset breast cancer

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**Summary**

The NOD2 gene has been associated with susceptibility to Crohn's disease, and more recently with carcinoma of the colon as well. NOD2 is involved in the inflammatory response and the activation of the NFkB pathway. The range of cancer types associated with NOD2 has not been well studied. The 3020insC allele results in a truncated NOD2 protein and is present in approximately 7% of the population. We studied a possible association between the 3020insC allele of the NOD2 gene and breast cancer using 462 cases and 1910 controls from Poland. Patients were diagnosed with invasive breast cancer at are of two Szczecin regional hospitals between 2002 and 2004. Pathology specimens were reviewed for histological subtype and for the presence of ductal carcinoma *in situ* (DCIS). Overall there was no association between breast cancer and NOD2 (OR = 1.1; $p = 0.76$), but significant associations were observed between the presence of the allele and early-onset breast cancer (OR = 1.9; $p = 0.01$) and between the allele and ductal breast cancer with an *in situ* component (OR = 2.2; $p = 0.006$).

## Introduction

The Nod2 protein is a component of the host defense system against intracellular pathogens and is expressed by monocytes, granulocytes and several epithelial cell types [1, 2]. Stimulation of NOD2-transfected cell lines with bacterial protein results in the activation of the nuclear factor kB (NFkB) pathway and deletion of NOD2 leads to enhanced NF-kB activity [2]. The most common mutation, 3020insC, leads to the introduction of a stop codon in exon 11 and generates a truncated NOD2 protein which is missing the last 33 amino acids [3]. This variant is associated with an increase in NFkB activity [2], and with an increased predisposition to Crohns' disease [4] and to colorectal cancer [5]. In order better to define the range of cancer types associated with the NOD2 3020insC mutation we studied the presence of this allele in 462 breast cancer patients and 1910 controls.

## Methods

Breast cancer cases were collected from two large hospitals in the Szczecin area (University Clinic Hospitals, Regional Oncology Hospital) between 2002 and 2004. Participation was requested of study subjects during outpatient clinic visits to the surgical and medical oncology clinics. Patients were consecutive, newly diagnosed cases of invasive breast cancer, unselected for age, sex or for family history. Cancers were classified according to age of diagnosis (<50 years or $\geq$50 years). A representative slide from each breast cancer specimen was reviewed by the reference pathologist (WD). Breast cancers were divided into subgroups according to histology and the presence or absence of DCIS. A family history was obtained through patient interview. A patient was considered to have a positive family history of breast cancer if there was a first- or second-degree relative reported to have had breast cancer diagnosed at any age. In general, breast cancers in relatives were not confirmed by pathology report.

The control group has been described previously [5]. It includes 910 newborn children from six hospitals throughout Poland (Szczecin, Białystok, Gorzów Wlkp., Katowice and Wrocław) in 2003 and 2004. It also contains 1000 patients selected at random from the patient rolls of three family doctors practicing in the Szczecin region.

DNA was extracted from peripheral blood lymphocytes for all cases and controls. The presence of the 3020insC was detected using the method of Ogura et al. [3] which is based on an allele-specific PCR assay. Allele specific primers are used to generate fragments of 319 and 214 bp representing the wild-type and mutant alleles respectively.

Statistical analysis included the comparison of the proportions of the prevalence of the allele in cases and controls. Odds ratios were generated from two-by-two tables and statistical significance was assessed using the (two-sided) Fisher exact test. Odds ratios were also generated by patient subgroups defined by age of diagnosis, histologic subtype, family history and the presence of DCIS.

## Results

The NOD2 3020insC allele was found in 7.3% of individuals in the Polish control population and in 8.0% of the breast cancer patients (OR = 1.1; $p = 0.6$) (Table 1). However, the allele was found in 13.2% of the women diagnosed with breast cancer before the age of 50 (OR = 1.9; $p = 0.01$) and in 14.3% of the patients with breast cancer containing an *in situ* component (OR = 2.1; $p = 0.009$). A modest association was also found in women with a family history of cancer (OR = 1.6) but this was not statistically significant ($p = 0.15$). There was no association with the NOD2 mutation and any particular histologic subtype of cancer. DCIS was present in 27% of the invasive breast cancers, in approximately equal proportions in women diagnosed before age 50 (23%) and after age 50 (27%). The presence of DCIS and age of diagnosis were independent risk factors for the presence of a mutation. Among women under age 50, the presence of the NOD2 allele was associated with a fivefold increase in the risk of breast cancer with DCIS (OR = 4.7; $p = 0.0003$).

## Discussion

We have previously reported that the NOD2 gene is associated with an increased risk of colon cancer [5]. We now believe that the gene may also be responsible for a proportion of cases of breast cancer as well. The lifetime risk of cancer associated with a NOD2 mutation is not expected to be high – the risk of breast cancer to age 50 is approximately 1% and we estimate that this incidence is doubled in carriers of the deleterious allele (and not increased thereafter) – but the mutant allele is relatively frequent in the general population (7.3% in Poland). In contrast, the CHEK2 1100delC allele is also associated with a twofold increase in breast cancer risk but is approximately 10 times less frequent in European populations [6].

Cases were recruited from the Szczecin region, which is populated by ethnic Poles who immigrated to the region from throughout Poland after the second world war, when German residents were relocated elsewhere. Our control group was drawn both from the adult population of Szczecin and from newborns in five Polish cities. However, the frequency of the 3020insC allele was similar in the newborns (7.8%) and in the adult population (6.9%) and there was no statistical difference in the mutant allele frequency in the newborns recruited from the Szczecin metropolitan region (8.2%) or from other Polish cities (7.3%). It is possible that there are minor differences in the ethnic distribution of our cases and controls, but more importantly, our association study benefits from the general homogeneity of the Polish population, which is much less ethnically diverse than the populations of North America or of most western European countries. This ethnic homogeneity has been exploited in several genetic studies in the past [7, 8]. This allele is present in other European countries and it will be of interest to see if our findings are replicated elsewhere.

Ductal carcinoma *in situ* may occur as an isolated lesion or in conjunction with invasive breast cancer. DCIS is widely regarded as a precursor lesion for some forms of breast cancer, and, if left untreated there is a high probability that DCIS will be followed by invasive breast cancer [9]. A family history of breast cancer is a risk factor for DCIS [10] and the risk of contralateral breast cancer is elevated among women following a diagnosis of DCIS [11]. These observations suggest that DCIS may have a hereditary component, but to date the

*Table 1.* Association of NOD2 3020insC mutation and selected types of cancer

| | Number tested | Number positive | Prevalence of 3020insC (%) | Odds ratio | *p*-Value |
|---|---|---|---|---|---|
| All Breast | 462 | 37 | 8.0 | 1.1 | 0.6 |
| <50 years | 159 | 21 | 13.2 | 1.9 | 0.01 |
| ≥50 years | 303 | 16 | 5.3 | 5.3 | 0.2 |
| Familial | 114 | 13 | 11.4 | 1.6 | 0.2 |
| Non-familial | 348 | 24 | 6.9 | 0.9 | 0.9 |
| Ductal | 372 | 30 | 8.1 | 1.1 | 0.7 |
| Lobular | 56 | 5 | 8.9 | 1.2 | 0.6 |
| Other | 32 | 2 | 6.2 | 0.8 | 0.9 |
| With DCIS | 126 | 18 | 14.3 | 2.1 | 0.008 |
| Without DCIS | 336 | 19 | 5.7 | 0.76 | 0.3 |
| Controls | 1910 | 140 | 7.3 | 1.0 | – |

genes responsible for this are unknown. DCIS does not feature prominently in the known breast cancer syndromes. Although it is often seen in families with BRCA2 mutations it is in fact seen less often than expected on its own [12] or in conjunction with invasive breast cancer in families with BRCA1 mutations [13]. Our study suggests that heterozygous inactivation of NOD2 through a frameshift mutation may be risk factor for breast cancer and for DCIS. The specificity of this association with breast cancers with an *in situ* component may represent a relevant biologic subgroup, but may be due to chance variation. Our sample size is small and this observation needs to be confirmed in a larger number of patients. Furthermore it will be of interest to study whether or not NOD2 predisposes to DCIS in the absence of invasive breast cancer.

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