Gene 587 (2016) 169–172

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

The c.657del5 variant in the *NBN* gene predisposes to pancreatic cancer

Marianna Borecka [a], Petra Zemankova [a], Filip Lhota [a], Jana Soukupova [a], Petra Kleiblova [a], Michal Vocka [b], Pavel Soucek [c], Ivana Ticha [d], Zdenek Kleibl [a], Marketa Janatova [a,*]

[a] Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic
[b] Department of Oncology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic
[c] Department of Toxicogenomics, National Institute of Public Health, Prague, Czech Republic
[d] Department of Pathology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form 22 April 2016
Accepted 29 April 2016
Available online 2 May 2016

Keywords:
Pancreatic ductal adenocarcinoma
The *NBN* gene
Inherited risk

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is the sixth most frequent cancer type in the Czech Republic with a poor prognosis that could be improved by an early detection and subsequent surgical treatment combined with chemotherapy. Genetic factors play an important role in PDAC risk. We previously identified one PDAC patient harboring the Slavic founder deleterious mutation c.657del5 in the *NBN* gene, using a panel next-generation sequencing (NGS). A subsequent analysis of 241 unselected PDAC patients revealed other mutation carriers. The overall frequency of c.657del5 in unselected PDAC patients (5/241; 2.07%) significantly differed from that in non-cancer controls (2/915; 0.2%; P = 0.006). The result indicates that the *NBN* c.657del5 variant represents a novel PDAC-susceptibility allele increasing PDAC risk (OR = 9.7; 95% CI: 1.9 to 50.2). The increased risk of PDAC in follow-up recommendations for *NBN* mutation carriers should be considered if other studies also confirm an increased frequency of c.657del5 carriers in PDAC patients from other populations.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the sixth most frequent cancer type (with an incidence of 19.6/100,000 persons in 2013) and the fifth most frequent cause of cancer death in the Czech Republic (www.svod.cz). The prognosis of PDAC is poor with a 5-year survival of 7% and a median survival of 6 months (Siegel et al., 2015). Early detection and subsequent surgical treatment combined with chemotherapy can improve the 5-year survival up to 40% (Nakao et al., 2006). While population screening is not rational due to the low PDAC incidence, the identification of high-risk individuals, who may benefit from the available screening methods, is desirable.

A genetic predisposition is the major endogenous risk factor of PDAC development, together with chronic pancreatitis and diabetes mellitus (Becker et al., 2014). It has been estimated that 5–10% of PDAC patients have a positive family PDAC history. The genetic basis of most familial PDAC cases has not been explained yet; however, several PDAC-susceptibility genes have been identified, including genes (*BRCA1*, *BRCA2*, *PALB2*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *STK11*, *APC*, *CDKN2A*) associated with hereditary cancer syndromes (reviewed in (Becker et al., 2014)). The protein products of numerous PDAC-susceptibility genes are directly involved in DNA repair and the DNA damage response. The most prevalent mutations have been identified in *BRCA2* (up to 6% of patients and increasing PDAC risk 3.5-fold (Couch et al., 2007)) and *PALB2* (3% of patients (Jones et al., 2009)). Their protein products share a common functional role in the DNA double-strand break (DDSB) repair. The *NBN* gene encodes nibrin, a protein participating in the formation of the multiprotein MRN (*MRE11-RAD50-NBN*) complex, an inevitable sensor of DNA damage in the DDSB repair (Carney et al., 1998). Biallelic *NBN* mutations predispose to the autosomal recessive Nijmegen-breakage syndrome (NBS) characterized by chromosomal instability and an increased risk of lymphoid malignancies and other cancers (Varon et al., 1998). Heterozygous *NBN* mutations predispose to breast cancer (BC) (Gorski et al., 2003), non-Hodgkin lymphoma (Steffen et al., 2006), and prostate cancer (Cybulski et al., 2013); however, their role in PDAC predisposition has not been studied yet. The most frequent pathogenic mutation in NBS patients and *NBN*-associated cancers is the recurrent Slavic founder mutation c.657del5 (c.657_661delACAAA) (Varon et al., 2000).

The next-generation sequencing (NGS) technology introduced analyses of large gene collections into genetic analyses in patients

Abbreviation: PDAC, pancreatic ductal adenocarcinoma; BRCA1, breast cancer gene 1; BRCA2, breast cancer gene 2; PALB2, partner and localizer of BRCA2; MLH1, homolog of *Escherichia coli* MutL 1; MSH2, homolog of *E. coli* MutS 2; MSH6, homolog of *E. coli* MutS 6; PMS2, postmeiotic segregation increased; STK11, serine/threonine protein kinase 11; APC, adenomatous polyposis coli gene; CDKN2A, cyclin-dependent kinase inhibitor 2A; DDSB, DNA double-strand break; NBN, nibrin; MRN, MRE11/RAD50/NBN complex; NBS, Nijmegen breakage syndrome; NGS, next-generation sequencing; FFPE, formalin-fixed paraffin-embedded; HRM, high resolution melting; FET, Fischer's exact test; OR, odds ratio; CI, confidence interval; BC, breast cancer; CHEK2, checkpoint kinase 2; PARP, poly(ADP-ribose) polymerase.

* Corresponding author at: Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University in Prague, U Nemocnice 5, 128 53 Prague 2, Czech Republic.
E-mail address: mjana@lf1.cuni.cz (M. Janatova).

http://dx.doi.org/10.1016/j.gene.2016.04.056
0378-1119/

with cancer susceptibility. Among others, *NBN* is routinely analyzed in many cancer gene sequencing panels. Recently, we have performed a study of germline variants influencing the breast cancer susceptibility in high-risk breast cancer patients using the custom panel NGS (Lhota et al., 2016). We subsequently used the identical approach for the analysis of pancreatic cancer predisposition in a PDAC patient from multiple cancer family. We identified the c.657del5 germline mutation in the *NBN* gene in this patient. Therefore, we aimed to determine the frequency of c.657del5 in unselected Czech PDAC patients.

2. Materials and methods

2.1. Panel NGS analysis in a patient with pancreatic ductal adenocarcinoma

In order to identify possible germline pathogenic variant in PDAC-susceptibility genes, we performed custom panel NGS targeting 581 genes in a PDAC patient from multiple cancer family (Fig. 1). The NGS and bioinformatics analysis was performed as described previously (Lhota et al., 2016) and revealed germline c.657del5 *NBN* variant. The mutation was confirmed by Sanger sequencing from independent PCR amplified blood DNA sample. The presence of the c.657del5 *NBN* variant in deceased proband's sister with gastric cancer (Fig. 1) was analyzed in DNA isolated from FFPE tumor tissue using the Cobas DNA Sample Preparation Kit (Roche).

2.2. Patients with pancreatic ductal adenocarcinoma

We genotyped c.657del5 *NBN* variant in blood-isolated DNA samples from 241 unselected, histopathologically-verified PDAC patients, which included 152 samples from the National Institute of Public Health [median age at diagnosis: 63 years (ranged 40–82); 59 females] and 89 samples from the Department of Oncology, General University Hospital in Prague [median age at diagnosis: 64 years (ranged 38–84); 49 females]. Information about family history of cancer in c.657del5 carriers was gathered from medical records when available.

The control group included 915 non-cancer individuals and it had been described and genotyped previously. All patients and controls were of Slavic descent and of Czech origin. The study was approved by the local Ethical Committees and a written informed consent was obtained from all participants.

2.3. The NBN c.657del5 genotyping

The exon 6 of the *NBN* gene was analyzed by a high resolution melting (HRM; LightCycler 480; Roche) using HOT FirePol EvaGreen HRM Mix (Solis BioDyne). The primer sequences had been described previously (Mateju et al., 2012). The presence of c.657del5 was confirmed by sequencing.

2.4. Statistical analysis

The difference between groups was calculated using the Fisher exact test (FET).

3. Results

We analyzed a PDAC patient (diagnosed at 64 years) from multiple-cancer family and identified the c.657del5 *NBN* mutation using the panel NGS (Fig. 1). Except to this germline mutation, we found no other truncating variants in other known PDAC-susceptibility genes (*BRCA1*, *BRCA2*, *PALB2*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *STK11*, *APC*, *CDKN2A*). The presence of c.657del5 mutation was confirmed also in the proband's sister deceased from gastric cancer (Fig. 1).

In the subsequent analysis, we genotyped c.657del5 in other 241 unselected PDAC patients and found five mutation carriers among them (2.07%). Thus, the frequency of c.657del5 among PDAC patients was significantly higher than that in previously analyzed controls (2/915), suggesting that the carriers of c.657del5 have an increased risk of PDAC development (OR = 9.7; 95%CI: 1.9–50.2; $P_{FET} = 0.006$). A PDAC family history was documented in none of the five c.657del5 carriers from 241 unselected PDAC patients; however, one patient had family cancer history (a sister with gastric cancer), and another female patient suffered from a duplicity of BC (at 46 years) and PDAC

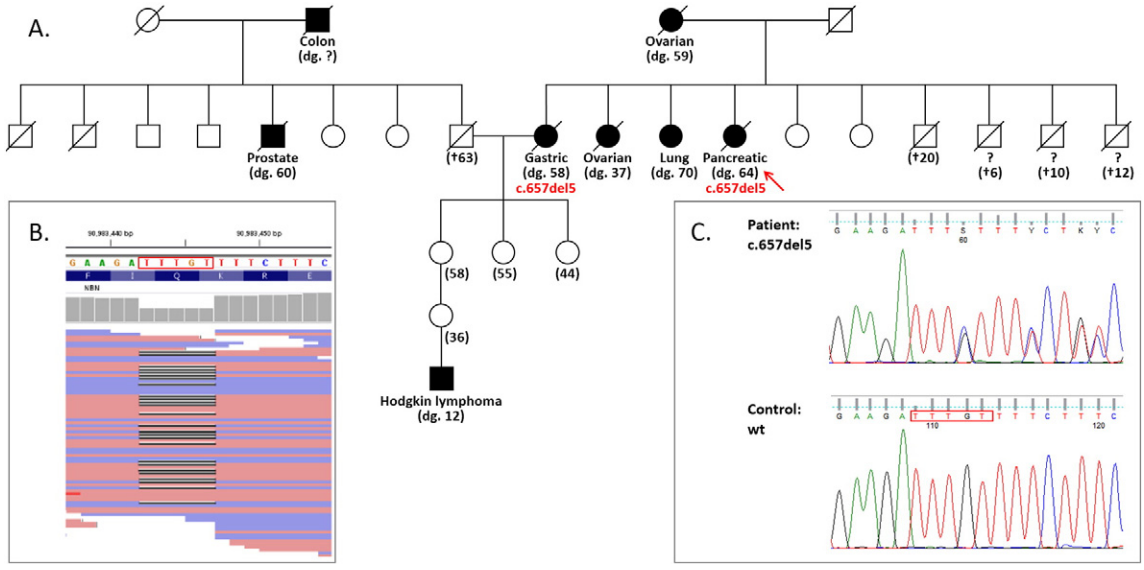

Fig. 1. Pedigree (A) of the multiple cancer family showing the proband with PDAC (indicated by an arrow) and her sister, both carrying c.657del5. DNA samples from other relatives were not available for genotyping. The ages of cancer diagnoses (dg.) or cessation (†) are indicated in the pedigree. The deletion of five nucleotides (TTTGT from reverse strand) is highlighted by a red frame in NGS analysis (B), confirmed by Sanger sequencing (C).