available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Heterozygous carriers of the I171V mutation of the NBS1 gene have a significantly increased risk of solid malignant tumours

Jerzy Nowak[a,*], *Maria Mosor*[a], *Iwona Ziółkowska*[a], *Malgorzta Wierzbicka*[b], *Monika Pernak-Schwarz*[a], *Marta Przyborska*[a], *Krzysztof Rożnowski*[b], *Andrzej Pławski*[a], *Ryszard Słomski*[a], *Danuta Januszkiewicz*[a,b]

[a]*Institute of Human Genetics, Polish Academy of Sciences, 60-479 Poznań Strzeszyńska 32, Poland*
[b]*University of Medical Sciences, Poznań, Poland*

ARTICLE INFO

Article history:
Received 7 January 2008
Accepted 8 January 2008
Available online 15 February 2008

Keywords:
I171V mutation
NBS1 gene
Cancer predisposition

ABSTRACT

Homozygous mutation 657del5 within the NBS1 gene is responsible for the majority of Nijmegen breakage syndrome (NBS) cases. NBS patients are characterised by increased susceptibility to malignancies mainly of lymphoid origin. Recently it has been postulated that heterozygous carriers of 657del5 NBS1 mutation are at higher risk of cancer development. The aim of the study was to analyse the frequency of I171V mutation in NBS1 gene in 270 women with breast cancer, 176 patients with larynx cancer, 81 with second primary tumours of head and neck, 131 with colorectal carcinoma and 600 healthy individuals. I171V mutation was present in 17 cancer patients compared with only one in healthy individuals. This constitutes 2.58% in studied patients with malignancies and 0.17% in the control group ($P = 0.0002$; relative risk 1.827; odds ratio 15.886; 95% confidence interval 2.107–119.8). Since DNA was isolated from non malignant cells, all mutations found in cancer patients appeared to be of germinal origin. It can be concluded that NBS1 allele I171V may be a general susceptibility gene in solid tumours.

1. Introduction

The NBS1 gene responsible for Nijmegen breakage syndrome (NBS) has been identified on chromosome band 8q21.[1] Homozygous mutation 657del5 within the NBS1 gene is responsible for the majority of NBS cases.[1,2] Patients with NBS in addition to typical clinical symptoms are characterised by increased susceptibility to malignancies mainly of lymphoid origin.[1] More then 50% of NBS patients developed lymphomas, leukaemias, glioma or medulloblastoma.[1] Recently it has been postulated that heterozygous carriers of 657del5 NBS1 mutation are at higher risk of cancer development.[3,4] In addition to the most common 657del5 mutation, several other alternations in NBS1 gene have been described. Among them, 657del5 and R215W heterozygous mutations have been observed in higher frequency in cancer patients.[5–8] However, another study indicated that NBS1 657del5 allele may contribute only to a limited fraction of breast cancer cases[9,10] and non-Hodgkin's lymphoma.[11,12] Another heterozygous missense mutation in NBS1 gene, leading to the I171V substitution, has been suggested to be involved in the pathogenesis of acute lymphoblastic leukaemia (ALL).[13,14] On the contrary, Taylor et al. did not detect this variant of NBS1 either in any of 131 children with leukaemias or in 332 normal cord blood samples.[15] However, a recently described I171V homozygous missense mutation in the NBS1 gene associated with aplastic anaemia strongly suggests the contribution of this mutation to genomic instability.[16]

* *Corresponding author*: Tel.: +48618221312; fax: +48618233235.
E-mail address: nowakjs@man.poznan.pl (J. Nowak).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.01.006

To examine the role of the I171V mutation in malignancies, 270 women with breast cancer, 176 patients with larynx cancer, 81 with second primary tumours of head and neck, 131 with colorectal cancer and 600 healthy individuals have been tested.

2. Materials and methods

Peripheral blood samples from 270 women with breast cancer, 176 patients with larynx cancer, 81 patients with second primary tumours of head and neck, 131 with colorectal cancer were collected after histopathological examination of the tumour. The detailed localisation and histopathological findings of 81 patients with second primary tumours of head and neck is presented in Table 1. 600 anonymous blood samples collected on Guthrie cards drawn from the newborn screening programme were used as a control group. All the patients and population controls came from the same geographical region. The study was approved by the Ethics Committee of the Medical University in Poznań. DNA was extracted using a DNA extraction kit (Qiagen, GmbH, Germany). From Guthrie cards, DNA was isolated by boiling at 95 °C for 90 min. All samples were analysed by PCR-single strand conformation polymorphisms for exon 5 of NBS1 gene and by restiction analysis of PCR product using MundI (MfeI) enzyme (Fermentas). Primer sequences for 5 exon were as follows: Ex 5 F-TTA TGG ATG TAA ACA GCC TC, Ex 5 R-TAC CGA ACT ATA ACA CAG CA. The samples showing shifts on nondenaturing polyacrylamide gels or giving additional band after MundI digestion, were directly sequenced (ABI PRISM 377 DNA Sequencer, Applied Biosystems). The detailed procedure for the I171V mutation in NBS1 gene has been described in detail previously.[14]

3. Results and discussion

DNA samples from 270 women with breast cancer, 176 patients with larynx carcinoma, 81 patients with second primary tumours of head and neck localisation, 131 patients with colorectal cancer and 600 healthy individuals were tested for the I171V mutation in NBS1 gene. I171V mutation was present in 17 cancer patients compared with only one in healthy individuals. This constitutes 2.58% in studied patients with malignancies and 0.17% in the control group ($P = 0.0002$; relative risk 1.827; odds ratio 15.886; 95% confidence interval 2.107–119.8). The highest I171V mutation prevalence (6.17%) was found in second primary tumours of head and neck. Detailed incidence of I171V mutation in four groups of patients with various malignancies is presented in Table 2. The incidence of I171V mutation NBS1 gene in each patient group differed significantly in comparison to the controls.

To find out if the I171V mutation of NBS1 gene is cancer predisposing, we compare age of onset in carriers and non-carriers of I171V mutation. In Table 3 we reported median age and age ranges at diagnosis of patients, carriers and non-carriers of I171V mutation, from all groups. Although no significant difference has been found, a tendency of earlier age of onset in I171V mutation carriers was observed.

Since DNA was isolated from non malignant cells, all mutations found in cancer patients appeared to be of germinal origin. In patients as well as in healthy individuals only heterozygous I171V mutation was identified.

The high incidence of I171V mutation NBS1 gene found in the patients strongly suggests that it is etiologically related to cancer development. The I171V alternation occurs in the breast cancer carboxyl-terminal (BRCT) nibrin domain.[13] This domain is highly conserved in proteins involved in DNA repair and cell-cycle checkpoints playing significant role in double-strand DNA breaks. It is very likely that I171V alternation of BRCT domain changes the function of nibrin. Therefore, the involvement of I171V NBS1 gene mutation in cancer pathogenesis is very esential as suggested by Varon et al., who found four cases with that mutation among 47 children with first relapse of ALL.[13] In studies of Shimada

Table 1 – Localisation and histopathological findings of 81 patients with second primary malignant tumours of head and neck

	Primary tumour		Secondary tumour	
	Histopathology	Localisation	Histopathology	Localisation
I171V mutation carriers $n = 5$	Carcinoma planoepitheliale $n = 3$	Larynx $n = 2$	Carcinoma planoepitheliale $n = 5$	Larynx $n = 3$
		Lung $n = 1$		Lung $n = 1$
	Lymphoma $n = 1$	Tonsil $n = 1$		Tonsil $n = 1$
	Adenolymphoma $n = 1$	Tonsil $n = 1$		
I171V mutation non-carriers $n = 76$	Carcinoma planoepitheliale $n = 64$	Head and neck localisation (larynx, nose, parotid gland, tonsil, tongue, soft palate, lip, auricle) $n = 64$	Carcinoma planoepitheliale $n = 71$	Head and neck localisation (larynx, nose, parotid gland, tonsil, tongue, soft palate, lip, auricle) $n = 71$
	Carcinoma spinocellulare $n = 7$	Skin of face $n = 5$	Carcinoma spinocellulare $n = 5$	Skin of face, nose $n = 5$
		Ear $n = 2$		
	Sarcoma $n = 3$	Mandible $n = 3$		
	Lymhoma $n = 2$	Tonsils $n = 2$		

Table 2 – Analysis of I171V mutation incidence in various solid tumours and in the control group

	Breast cancer (*n* = 270)	Larynx cancer (*n* = 176)	Second primary tumours of head and neck localisation (*n* = 81)	Colorectal cancer (*n* = 131)	Control (*n* = 600)
I171V mutation carriers number	5	4	5	3	1
Percentage	1.85%	2.27%	6.17%	2.29%	0.17%
***p* value**	0.013	0.011	0.0001	0.0196	
Relative risk	2.717	3.586	7.401	4.26	
Odds ratio	11.302	13.93	39.408	14,39	
95% confidence interval	1.313–97.261	1.546–125.52	4.541–341.98	2.367–7.664	

For statistical analysis, Fisher's exact test has been used (GraphPad Prism, ver. 4.03). Studied groups were compared to the control.

Table 3 – Median age and age ranges of patient carriers and non-carriers of I171V mutation

Study group	I171V mutation carriers	I171V mutation non-carriers	Statistics
Breast cancer	*n* = 5	*n* = 265	NS
Range	41–62 years	31–75 years	
Median	49.4 years	52.4 years	
Larynx cancer	*n* = 4	*n* = 172	NS
Range	51–74 years	41–81 years	
Median	61 years	60 years	
Second primary tumours	*n* = 5	*n* = 76	NS
	1st tumour	1st tumour	
Range	30–84 years	49–77 years	
Median	56 years	58 years	
	2nd tumour	2nd tumour	NS
Range	43–84 years	54–78 years	
Median	60 years	61 years	
Colorectal cancer	*n* = 3	*n* = 128	NS
Range	45–68 years	24–83 years	
Median	56 years	60 years	

For statistical analysis, the Mann–Whitney test has been used (GraphPad Prism, ver. 4.03).

et al. and Taylor et al. none of the patients with ALL (number of cases 29 and 155 respectively) were found to be positive for I171V NBS1 gene mutation.[15,16] In the above studies only Shimada et al. found five individuals with heterozygous I171V NBS1 gene mutation among 413 normal controls.[16] High frequency of I171V NBS1 gene mutation found in the studied cancer patients strongly suggests the involvement of this mutation in carcinogenesis. It is very interesting that this mutation is characteristic for many types of solid tumours. On the basis of the obtained results it can be concluded that I171V mutation in NBS1 gene is associated with predisposition to malignancies and NBS1 allele I171V may be a general cancer susceptibility gene.

Conflict of interest statement

None declared.

Acknowledgement

Financial support: N 407 2854 34 and 2P05C 039 29.

REFERENCES

1. The International Nijmegen Breakage Syndrome Study Group. Nijmegen breakage syndrome. Arch Dis Child 2000;82:400–6.
2. Varon R, Vissinga C, Platzer M, et al. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* 1998;**93**:467–76.
3. Seemanova E. An increased risk for malignant neoplasms in heterozygotes for a syndrome of microcephaly, normal intelligence, growth retardation, remarkable facies, immunodeficiency and chromosomal instability. *Mutat Res* 1990;**238**:321–4.
4. Steffen J, Varon R, Mosor M, et al. Increased cancer risk of heterozygotes with NBS1 germline mutations in Poland. *Int J Cancer* 2004;**111**:67–71.
5. Gorski B, Debniak T, Masojc B, et al. Germline 657del5 mutation in the NBS1 gene in breast cancer patients. *Int J Cancer* 2003;**106**:379–81.
6. Gorski B, Cybulski C, Huzarski T, et al. Breast cancer predisposing alleles in Poland. *Breast Cancer Res Treat* 2005;**92**:19–24.
7. Debniak T, Gorski B, Cybulski C, et al. Germline 657del5 mutation in the NBS1 gene in patients with malignant melanoma of the skin. *Melanoma Res* 2003;**13**:365–70.
8. Cybulski C, Gorski B, Debniak T, et al. NBS1 is a prostate cancer susceptibility gene. *Cancer Res* 2004;**64**:1215–9.
9. Plisiecka-Halasa J, Dansonka-Mieszkowska A, Rembiszewska M, Bidzinski M, Steffen J, Kupryjanczyk J. Nijmegen breakage syndrome gene (NBS1) alterations and its protein (nibrin) expression in human ovarian tumours. *Ann Hum Genet* 2002;**66**:353–9.
10. Buslov KG, Iyevleva AG, Chekmariova EV, et al. NBS1 657del5 mutation may contribute only to a limited fraction of breast cancer cases in Russia. *Int J Cancer* 2005;**114**:585–9.
11. Soucek P, Gut I, Trneny M, et al. Multiplex single-tube screening for mutations in the Nijmegen Breakage Syndrome (NBS1) gene in Hodgkin's and non-Hodgkin's lymphoma patients of Slavic origin. *Eur J Hum Genet* 2003;**11**:416–9.
12. Cerosaletti KM, Morrison VA, Sabath DE, Willerford DM, Concannon P. Mutations and molecular variants of the NBS1

gene in non-Hodgkin lymphoma. *Genes Chromosomes Cancer* 2002;**35**:282–6.
13. Varon R, Schoch C, Reis A, et al. Mutation analysis of the Nijmegen breakage syndrome gene (NBS1) in nineteen patients with acute myeloid leukemia with complex karyotypes. *Leuk Lymphoma* 2003;**44**:1931–4.
14. Mosor M, Ziolkowska I, Pernak-Schwarz M, Januszkiewicz-Lewandowska D, Nowak J. Association of the heterozygous germline I171V mutation of the NBS1 gene with childhood acute lymphoblastic leukemia. *Leukemia* 2006;**20**:1454–6.
15. Taylor GM, O'Brien HP, Greaves MF, Ravetto PF, Eden OB. Correspondence re: R. Varon et al. Mutations in the Nijmegen breakage syndrome gene (NBS1) in childhood acute lymphoblastic leukemia. Cancer Res, 2001;61:3570-3572. Cancer Res, 2003;63:6563-6564.
16. Shimada H, Shimizu K, Mimaki S, et al. First case of aplastic anemia in a Japanese child with a homozygous missense mutation in the NBS1 gene (I171V) associated with genomic instability. *Hum Genet* 2004;**115**:372–6.