Increased risk of larynx cancer in heterozygous carriers of the I171V mutation of the *NBS1* gene

Iwona Ziólkowska,[1,3] Maria Mosor,[1] Malgorzata Wierzbicka,[2] Malgorzata Rydzanicz,[1] Monika Pernak-Schwarz[1] and Jerzy Nowak[1]

[1]Department of Molecular Pathology, Institute of Human Genetics, Polish Academy of Sciences, Strzeszyñska St 32, Poznan 60-479; [2]Department of Otolaryngology and Laryngeal Oncology, K. Marcinkowski University of Medical Sciences, Przybyszewskiego St 49, Poznan 60-355, Poland

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The high incidence of multiple primary tumors (MPT) is a significant problem in head and neck tumor treatment. Recent studies suggest that carriers of heterozygous mutations in the *NBS1* gene have an increased risk of malignant tumor development. The aim of our research was to assess the frequency of *NBS1* mutations in patients with larynx cancer only (LC) and with MPT. The MPT group consisted of patients with one cancer localized to the larynx (primary or second) and another at another site. DNA from 175 patients with LC and 93 patients with MPT was analyzed using the single-strand conformation polymorphism method and direct sequencing. We found nine carriers of the I171V mutation among these 268 cancer patients and only one carrier among 500 population controls (0.2%). Four carriers of the I171V mutation were detected among 175 LC patients (2.3%) and five among 93 patients with MPT (5.4%). The frequencies of the I171V mutation carriers in LC and MPT patients were significantly higher than in controls (odds ratio [OR] = 11.7, confidence interval [CI] 1.3–105.2, $P = 0.0175$ and OR = 28.35, CI 3.27–245.7, $P = 0.0005$, respectively). In one individual with LC, a novel molecular variant, c.1222 A > G (p.K408E), was identified. No carriers of R215W or 657del5 *NBS1* mutations were found in the present study. These findings imply that heterozygous carriers of the I171V mutation are prone to the development of larynx cancer and may, in addition, display an increased risk of second tumors at other sites. (*Cancer Sci* 2007; 98: 1701–1705)

Despite recent advances in the diagnosis and treatment of head and neck squamous cell carcinoma (HNSCC), the survival rate of patients with these tumors is not improving. One of the reasons for this is the frequent development of second primary tumors (SPT). Among HNSCC patients, the frequency of SPT occurrence ranges from 12 to 30% with a constant rate of 2–3% per year for patients who survive for more than 10 years.[1–3] The first tumor is responsible for 15% of patient deaths, whereas the second tumor is the cause of death in 71% of cases. Squamous cell carcinoma is the predominant histological type of the second tumor.[4]

In the etiology of multiple primary tumors (MPT) of the head and neck, tobacco and alcohol abuse ('condemned mucosa theory') are essential but are not the only causal factors.[5] It is supposed that genetic factors play a great role in the occurrence of the first tumor and, in addition, predispose the patient to the development of a second primary tumor. Because of reduced DNA repair capacity in patients who had two or more independent malignancies,[6] it has been suggested that alterations in DNA repair genes may play a significant role in head and neck tumor development.

The *NBS1* gene belongs to a group of double-strand break repair genes and is located on chromosome band 8q21.3.[7–9] Biallelic mutations in the *NBS1* gene are responsible for Nijmegen breakage syndrome (NBS, OMIM 251260), classified to a group of chromosomal instability syndromes. NBS is a rare autosomal recessive disorder, occurring mainly in central and eastern Europe.[10] The most common is the homozygous 657del5 mutation, observed in 90% of NBS patients. The protein product of the *NBS1* gene, nibrin (p95, NBS1), is a member of the MRE11–RAD50–nibrin complex,[7] which is involved in DNA double-strand break repair by homologous recombination or non-homologous end joining, meiotic recombination and the DNA damage response.[7,11] The MRE11–RAD50–nibrin complex is also required for activation of the damage checkpoint kinase ataxia telangiectasia mutated (ATM), which is involved in activating DNA damage signaling pathways and cell cycle checkpoints.[12] It is known that almost 40% of patients with NBS have developed a malignancy (mainly lymphomas) before the age of 21 years.[13] In 1990, Seemanova suggested that *NBS1* heterozygotes might have an elevated cancer risk, as evident by the high incidence of malignancies in relatives of NBS patients.[14] Recent findings show that besides spontaneous chromosome instability,[15,16] cells from *NBS1* gene mutation carriers are characterized by a distinct gene expression phenotype.[17] Heterozygous mutations in the *NBS1* gene have been described in various groups of cancer patients, for example, patients with acute lymphoblastic leukemia (ALL),[18,19] non-Hodgkin's lymphoma (NHL),[20] and in cancer cell lines.[21] Recent studies strongly suggest that heterozygous 657del5 mutation carriers have an elevated risk of malignant tumor development, especially of melanoma,[22,23] colon and rectum cancer,[23] prostate cancer,[24] ovarian cancer,[25] breast cancer[26,27] and NHL.[28,29]

The aim of our research was to assess whether mutations in the *NBS1* gene may contribute to the development of larynx cancer (LC) and MPT of the head and neck.

Materials and Methods

Patients and controls. Blood samples were collected from Polish patients: 93 with MPT with one malignancy (primary or second) localized to the larynx and 175 with larynx cancer only (LC). All patients were treated in the Department of Otolaryngology and Head and Neck Oncology, K. Marcinkowski University of Medical Sciences, Poznan.

All patients included in the MPT group fulfilled all of the criteria proposed by Warren and Gates[30] and accepted by the International Agency for Research of Cancer. These criteria are indispensable for distinguishing a SPT at a neighboring anatomical site from a local recurrence: (1) each of the tumors must present a definite picture of malignancy; (2) each must be distinct; and (3) the probability of one being a metastasis of the other must be excluded. The second criterion was precise: a 2-cm distance of unchanged mucosa distinguished SPT from local recurrence at the same or at an adjacent anatomical site. Finally, a time interval longer than 3 years between occurrence of the primary

[3]To whom correspondence should be addressed. E-mail: iwonus21@wp.pl

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Table 1. Characteristics of patients with multiple primary tumors (*n* = 93)

Factor	Index tumors	Second tumors	All tumors
Age (years)			
≤60			45 (48.4)
>60			48 (51.6)
Sex			
Female			9 (9.7)
Male			84 (90.3)
Localization			
Larynx	60 (64.5)	39 (41.9)	
Lung	9 (9.7)	10 (10.8)	
Tonsil	4 (4.3)	9 (9.7)	
Tongue	4 (4.3)	1 (1.1)	
Colon or rectum	2 (2.2)	2 (2.2)	
Prostate	2 (2.2)	3 (3.2)	
Soft palate	1 (1.1)	2 (2.2)	
Kidney	1 (1.1)	1 (1.1)	
Urinary bladder	1 (1.1)	1 (1.1)	
Parotid gland	–	6 (6.5)	
Skin of the nose	–	4 (4.3)	
Esophagus	–	4 (4.3)	
Lip	–	3 (3.2)	
Thyroid gland	–	3 (3.2)	
Other	9 (9.7)	5 (5.3)	
Histological type			
Carcinoma planoepitheliale	73 (78.5)	65 (69.9)	
Adenocarcinoma	8 (8.6)	9 (9.7)	
Adenocarcinoma tubulare	4 (4.3)	–	
Carcinoma cystis bronchiogenes	2 (2.2)	2 (2.2)	
Melanoma malignum	2 (2.2)	–	
Cystadenolymphoma	–	5 (5.3)	
Carcinoma basocellulare	–	3 (3.2)	
Adenoma polymorphum	–	2 (2.2)	
Carcinoma papillare	–	2 (2.2)	
Carcinoma urotheliale	–	2 (2.2)	
Other	4 (4.3)	3 (3.2)	

Numbers in parentheses are percentages.

and secondary carcinomas is considered to be a criterion. Second primary tumors can be divided into two groups: synchronous, when MPT develop simultaneously or within 6 months of the first (i.e. index tumors), and metachronous, if they develop more than 6 months after the index tumor.

In all 93 MPT cases either the index or second tumor was LC. The characteristics of MPT patients are given in Table 1. The majority of them were adult males ($P < 0.0001$), age range from 23 to 84 years, mean age 57.3 years. The most common localization for the index (60 cases, 64.5%) and second (39 cases, 41.9%) tumors was the larynx. The first tumors described in Table 1 as 'other' were located in the jaw (two patients), and single cases were located in the oral cavity, paranasal sinus, pituitary gland, stomach, skin of the thigh and supraclavicular region, and one patient was treated successfully for lymphoma. The 'other' sites of the second tumor were single cases in the alveolar process, skin of the face, suprarenal gland, ovary and lymph node (NHL). The histological type of first and second tumors was predominantly squamous cell carcinoma (*carcinoma planoepitheliale*). The histological types described as 'other' were as follows for the index (first) tumor: *c. anaplasticum*, *c. clarocellulare*, *c. solidum* and *NHL*; and for the second tumor: *c. spinocellulare*, *carcinoid* and *lymphogranulomatosis maligna*. Six of the 93 patients with MPT developed three malignancies: a tumor of the lung (two patients) and, in single cases, of the larynx, uvula, prostate and testis.

Generally 195 anonymous, unutilized blood samples on Guthrie cards were used as controls. For exon 5, 500 Guthrie cards constituted the control group. The blood samples were collected during the newborn screening program for phenyloketonuria of the Wielkopolska province and were deposited at the Screening Research Laboratory in Poznan. The research protocol was approved by the Ethics Committee of the University of Medical Sciences in Poznan.

Methods. DNA from the 268 samples from MPT and LC patients was isolated from peripheral lymphocytes by proteinase K (Sigma, USA) digestion followed by phenol–chloroform extraction and ethanol precipitation. A piece of each Guthrie card was boiled at 95°C for 90 min for DNA extraction. All exons of the *NBS1* gene were amplified by polymerase chain reaction (PCR) under suitable conditions on an MJ PTC-200 (GMI, Minnesota, MN, USA) thermocycler. A set of 20 specific intronic primers flanking each exon of the *NBS1* gene was designed as described earlier.[19] The amplification reactions were carried out in a total reaction volume of 25 μL, containing 1 U *Taq* DNA Polymerase, 1× PCR buffer with 15 mM $MgCl_2$ (Eppendorf, Germany), 0.4 M of each dNTP (Sigma-Aldrich, Germany), 10 pmol of primers (Oligo, Poland) and 50–100 ng genomic DNA. All samples were analyzed by PCR–single-strand conformation polymorphism (SSCP). After mixing with the loading buffer and denaturation (95°C/5 min) the PCR product was separated on a 7% non-denaturing polyacrylamide gel under suitable conditions (gel with or without 5% glycerol, room temperature or 4°C). For DNA visualization, the silver-staining method was used. The substitution A > G at 511 (p.I171V) was investigated by PCR–restriction fragment length polymorphism (RFLP) analysis of the mutation with restriction enzymes, as described earlier.[19] Samples that showed an aberrant shift in either PCR-SSCP or PCR-RFLP were purified with a QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced directly on an ABI PRISM310 Sequencer with the use of a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA).

The significance of differences between studied groups was assessed using the χ^2 test or Fischer's exact test, depending on the variants' frequencies. Crude odds ratios (OR) were calculated and given with 95% confidence intervals (CI). The differences were considered significant if the value of the probability (*P*)

Table 2. Frequencies of heterozygous mutation and molecular variant carriers in patients with larynx cancer, multiple primary tumors and controls

Patients	I171V			D95N			K408E		
	n	OR (95% CI)	*P*	*n*	OR (95% CI)	*P*	*n*	OR (95% CI)	*P*
Larynx cancer	4/175	11.7 (1.295–105.2)	0.0175*	0/175	–	–	1/175	6.3 (0.25–156.5)	0.324
Multiple primary tumors	5/93	28.35 (3.27–245.7)	0.0005*	1/93	2.1 (0.13–34.11)	0.5423	0/93	–	–
Controls	1/500			1/195			0/195		

*Result statistically significant ($P \leq 0.05$). CI, confidence interval; OR, odds ratio.

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Table 3. Characteristic of heterozygous I171V mutation carriers among multiple primary tumor (MPT) patients

	MPT20	MPT68	MPT82	MPT83	MPT141
Birth year	1931	1932	1938	1925	1954
Age at index occurrence (years)	63	59	65	77	44
Age at second primary occurrence (years)	66	70	65	78	51
First tumor localization	Rectum	Lung L	Larynx	Lymphoma	Larynx
First tumor histology	*Adenocarcinoma tubulare*	*Carcinoma planoepitheliale*	*Carcinoma planoepitheliale*	*Non-Hodgkin lymphoma*	*Carcinoma planoepitheliale*
RTG	–	–	–	–	–
Chemotherapy	–	–	–	+	–
Second tumor localization	Larynx	Larynx	Lung R	Larynx	Parotid L
Second tumor histology	*Carcinoma planoepitheliale*	*Carcinoma planoepitheliale*	*Carcinoma planoepitheliale*	*Carcinoma planoepitheliale*	*Adenoma polymorphum*
Third tumor	–	+	–	+	+
Third tumor localization	–	Testis	–	Lung R	Oral cavity
Third tumor histology	–	Seminoma	–	*Carcinoma planoepitheliale*	*Carcinoma planoepitheliale*

L, left; R, right; RTG, radiation therapy.

did not exceed 0.05. In the case of polymorphisms the wild-type genotype served as a reference category. Mean ages of carriers and non-carriers were compared using the Mann–Whitney *U*-test.

Results

Among all 268 of the cancer patients, we detected one mutation and two distinct molecular variants of the *NBS1* gene (Table 2). We found nine carriers of the I171V substitution (c.511 A > G): five among MPT patients (5.4%, 5/93) and four among LC patients (2.3%, 4/175). Only one carrier of the I171V mutation was identified in the control group ($n = 500$). The frequencies of I171V mutation carriers in the MPT (OR = 28.35, CI 3.27–245.7, $P = 0.0005$) and LC (OR = 11.7, CI 1.3–105.2, $P = 0.0175$) groups were significantly higher than in controls. All of the I171V mutations were found in male patients. In the MPT group, the second tumor localization was different to the first tumor in all five patients who carried the I171V mutation. A detailed characterization of the MPT patients harboring the I171V mutation is presented in Table 3. The mean ages of the I171V mutation carriers and non-carriers in the LC group were similar (60.3 and 59.6 years, respectively, $P = 0.9701$). In the MPT group, the mean ages at diagnosis of the five I171V mutation carriers and non-carriers (61.6 and 57 years, respectively) did not differ significantly ($P = 0.3938$). The mean time intervals between the first and second tumor diagnosis in carriers and non-carriers were, respectively, 5.5 and 7.5 years. This difference was not significant ($P = 0.6931$).

In exon 3 of the *NBS1* gene, a G > A transition at position 283 (c.282G > A), leading to amino acid change p.D95N, was observed in one patient with MPT and in one individual in the control group.

A novel molecular variant, in exon 10 of the *NBS1* gene, was identified in one individual with a single tumor in the head and neck region (LC132). This transition A > G at position 1222 (c.1222 A > G) leads to substitution of the basic amino acid lysine with glutamic acid (p.K408E) in nibrin. The K408E variant was not observed in the control group. No carriers of the 657del5 (c.657–661delACAAA) and R215W mutations were found in the present study.

In single cases we found some previously described *NBS1* sequence variants c.930T > A (LC177), c.1489 A > G (MPT25), and additionally a novel rare sequence variant, in exon 15 of the *NBS1* gene: c.2202 A > G (MPT76). We also detected five intronic variant sequences among patients, flanking exon 7: IVS6–18G > A (c.703–18G > A), IVS6–29C > T (c.703–29C > T), IVS7 + 36G > A (c.896 + 36G > A), IVS8–42G > C (c.897–42G > C), and IVS15 + 88C > G (c.2234 + 88C > G). Some of these rare sequence variants were also found in the control group, but their frequencies in patients and controls did not differ significantly. We also calculated the frequencies of six polymorphic sites of the *NBS1* gene: 102G > A, 553G > C, IVS9 + 18C > T (c.1124 + 18C > T), 1197T > C, 2016 A > G, and IVS13–30 A > T (c.2071–30 A > T) (Table 4), and showed that the 553G > C GC and 2016 A > G AG genotypes were significantly more frequent in cancer patients than in controls.

Discussion

In the present study we found that the frequency of *NBS1* I171V mutation carriers, in the pooled group of LC and MPT cancer patients, was significantly higher than in population controls, which implies that this mutation contributes significantly to the overall incidence of larynx carcinoma. Carriers of the I171V mutation may also be prone to the development of second malignancies, as the frequency of this mutation among MPT patients was apparently higher than among patients with LC; however, the difference was not significant (OR = 2.43, CI 0.64–9.28, $P = 0.28$). The I171V substitution was identified for the first time by Varon *et al*. in five of 47 children with ALL.[18] They noticed that most of the children harboring the *NBS1* gene mutation had poor prognoses because of late relapses. The first homozygous I171V mutation was confirmed in a patient with aplastic anemia (AA).[31] Among 53 Japanese patients with hematological malignancies and nine with AA, only one girl (patient NCC56) carried this mutation. Furthermore, a higher frequency of chromosomal structural aberrations, which leads to genomic instability, was demonstrated by cytogenetic analyses in lymphoblastoid cell lines from patient NCC56 and her father (carrier of the I171V mutation). The pathogenic character of the I171V mutation is presumably connected with its occurrence in the BRCA1 carboxy-terminal (BRCT) domain of nibrin. The BRCT C-terminal domain is widely conserved in eukaryotic nuclear proteins related to the cell cycle, gene regulation and DNA repair.[32] The fork head-associated (FHA) and BRCT domains have a crucial role for both binding to histone γ-H2AX and for delocalization of the MRE11–RAD50 complex to the vicinity of the DNA damage site.[33] The synthesis of the truncated protein disturbs the function of the MRE11–RAD50–nibrin complex. Disturbances in DNA repair may lead to increased chromosome instability, which was detected in 65% of patients with one tumor in the head and neck region.[34]

Table 4. Genotype frequencies and logistic regression analysis (with odds ratios [OR] and 95% confidence intervals [CI]) of the *NBS1* gene polymorphic alleles in multiple primary tumors (MPT), larynx cancer (LC) and controls

Polymorphism	Genotype	Controls *n* (%)	MPT *n* (%)	OR (95% CI)	*P*	LC *n* (%)	OR (95% CI)	*P*
	GG	79 (41)	43 (46)	1.00†		81 (46)	1.00†	
102G > A	GA	92 (47)	38 (41)	0.78 (0.45–1.29)	0.31	65 (37)	1.45 (0.93–2.26)	0.10
	AA	24 (12)	12 (13)	0.92 (0.42–2.02)	0.83	29 (17)	0.85 (0.45–1.58)	0.61
	GG	82 (42)	33 (36)	1.00†		89 (51)	1.00†	
553G > C	GC	93 (48)	44 (47)	1.18 (0.68–2.02)	0.56	69 (34)	1.68 (1.08–2.62)	0.02*
	CC	20 (10)	16 (17)	1.99 (0.92–4.30)	0.08	26 (15)	0.83 (0.43–1.61)	0.59
	CC	80 (41)	35 (38)	1.00†		70 (40)	1.00†	
IVS9 + 18C > T	CT	90 (46)	41 (44)	1.04 (0.61–1.79)	0.88	64 (37)	1.23 (0.78–1.94)	0.37
	TT	25 (13)	17 (18)	1.55 (0.75–3.24)	0.24	41 (23)	0.53 (0.30–0.94)	0.04*
	TT	70 (36)	35 (38)	1.00†		75 (43)	1.00†	
1197T > C	TC	90 (46)	45 (48)	1.00 (0.58–1.72)	1.00	76 (43)	1.27 (0.81–1.98)	0.30
	CC	35 (18)	13 (14)	0.74 (0.35–1.58)	0.44	24 (14)	1.56 (0.85–2.89)	0.15
	AA	76 (39)	37 (40)	1.00†		89 (51)	1.00†	
2016 A > G	AG	92 (47)	43 (46)	0.96 (0.56–1.63)	0.88	60 (34)	1.80 (1.15–2.81)	0.01*
	GG	27 (14)	13 (14)	0.98 (0.46–2.14)	0.98	26 (15)	1.25 (0.65–2.26)	0.54
	AA	78 (40)	37 (40)	1.00†		88 (50)	1.00†	
IVS13–30 A > T	AT	95 (49)	46 (50)	1.02 (0.60–1.73)	0.94	59 (34)	1.82 (1.16–2.84)	0.01*
	TT	22 (11)	10 (10)	0.96 (0.41–2.23)	0.92	28 (16)	0.89 (0.47–1.68)	0.71

*Result statistically significant ($P = 0.05$); †Reference category.

We also found another heterozygous molecular variant, D95N, in one MPT patient. This variant was also identified in one individual with ALL in the German population (1/47).[18] However, in the British population, the D95N substitution was not found among 321 children with primary leukemia and lymphoma, but was detected in one of 332 normal cord blood samples.[35] Similarly, there was no carrier of this substitution among 91 patients with sporadic NHK in the US population versus two found in 154 control individuals.[20] Thus, it appears that the D95N variant does not have any impact on tumor risk, despite its occurrence in a functional part of nibrin (the FHA domain).

None of the 268 cancer patients carried the 657del5 mutation in exon 6 of the *NBS1* gene, suggesting that this mutation is not involved in the development of LC or MPT. The frequency of 657del5 mutation carriers is relatively high in Slavic populations, reaching 1/177 in southern and 1/162 in central regions of Poland.[10,23] In contrast to our study, three 657del5 mutation carriers with cancer, described by Steffen *et al*.,[23,27] had developed a second primary tumor. Recent findings for large groups of Polish patients strongly suggest the elevated risk of sporadic lymphoid malignancies (ALL 3/270 and NHL 2/212),[29] NHL (6/186), especially of the gastrointestinal tract,[28] and breast cancer (11/562)[27] in heterozygous 657del5 mutation carriers. However, the potential link between the 657del5 mutation and cancer risk could not be proven in a Slavic (Czech) population in patients with Hodgkin's/NHL[36] and in non-Slavic populations with low population frequencies of 657del5, for example, in German patients with NHL[37,38] or breast cancer,[39] in British children with primary leukemia and primary lymphoma,[35] and in Japanese patients with B-cell malignant lymphoma.[40]

Another mutation, c.643C > T (p.R215W), in exon 6 of the *NBS1* gene, was previously found in four samples among 1620 Slavic probands.[23] The carriers of the R215W mutation were described among patients with head and neck cancer (1/27), colon and rectum cancer (3/234)[23] and ALL (4/231).[35] No carriers of this rare mutation were found in our present study.

The frequencies of intronic sequence variants detected in the examined group of patients were similar to those observed in controls. Three of these variants (IVS6–29C > T, IVS8–42G > C and IVS15 + 88C > G) were previously reported in patients with NHL.[20] Similar to our results, there were no significant differences in the frequencies of these variants between cancer patients and population controls. The distributions of most of the polymorphic *NBS1* alleles among MPT and LC patients and controls (except for four genotypes at sites 553G > C, IVS9 + 18C > T, 2016 A > G and IVS13–30 A > T) did not differ between patients and controls. So far, only Zienolddiny *et al*.[41] reported that Finnish 553G > C homozygotes (p.E186E) had a decreased non-small-cell lung cancer risk. Thus, larger studies are needed to confirm the associations between the various *NBS1* polymorphic alleles and cancer risk.

It is possible that *NBS1* gene mutation carriers may show an increased cellular sensitivity to ionizing radiation and cytostatic drugs. Radiation therapy is used routinely in LC patients with primary tumors as primary or adjuvant treatment,[42] which may enhance the risk of development of a second tumor in carriers. Among our 93 patients with MPT, 27 (29%) were treated with irradiation, six (6.5%) with chemotherapy and two (2.2%) with both modalities before the diagnosis of the second tumor. This group included only one carrier (MPT83) of the I171V mutation who underwent chemotherapy. None of the carriers were treated with radiation. Thus, we could not prove in our study that radiotherapy or chemotherapy has an impact on second tumor occurrence in *NBS1* gene mutation carriers.

Recent findings suggest that the *NBS1* gene may have an impact on tumor treatment as a prognostic marker in cancers such as uveal melanoma[43] and HNSCC.[44] In particular, Yang *et al*. showed that expression of the *NBS1* gene was increased in 45% of patients with advanced HNSCC, making it an independent marker of adverse prognosis.[44]

In conclusion, our present study demonstrates that carriers of the I171V mutation of the *NBS1* gene have a significantly increased risk of larynx cancer and, in addition, may be predisposed to the development of second primary tumors of the larynx and at other sites. Further studies are needed to prove whether mutations in the *NBS1* gene contribute to cancer incidence in general.

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References

1 Gluckman JL, Crissman JD. Survival rates in 548 patients with multiple neoplasms of the upper aerodigestive tract. *Laryngoscope* 1983; **93**: 71–4.
2 Jones AS, Morar P, Phillips DE, Field JK, Husband D, Helliwell TR. Second primary tumors in patients with head and neck squamous cell carcinoma. *Cancer* 1995; **75**: 1343–53.
3 Kulczynski B, Pruszewicz M, Wierzbicka M. Incidence of second primary malignant neoplasms in patients treated for laryngeal cancer. *Otolaryngol Pol* 1995; **49** (Suppl 20): 322–8. (In Polish.)
4 Cianfriglia F, Di Gregorio DA, Manieri A. Multiple primary tumours in patients with oral squamous cell carcinoma. *Oral Oncol* 1999; **35**: 157–63.
5 Day GL, Blot WJ, Shore RE *et al*. Second cancers following oral and pharyngeal cancers: role of tobacco and alcohol. *J Natl Cancer Inst* 1994; **86**: 131–7.
6 Miller DG, Tiwari R, Pathak S, Hopwood VL, Gilbert F, Hsu TC. DNA repair and mutagen sensitivity in patients with triple primary cancers. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 321–7.
7 Carney JP, Maser RS, Olivares H *et al*. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double strand break repair to the cellular DNA damage response. *Cell* 1998; **93**: 477–86.
8 Matsurra S, Tauchi H, Nakamura A *et al*. Positional cloning of the gene for Nijmegen breakage syndrome. *Nat Genet* 1998; **19**: 179–81.
9 Varon R, Vissinga CH, Platzer M *et al*. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* 1998; **93**: 467–76.
10 Varon R, Seemanova E, Chrzanowska K *et al*. Clinical ascertainment of Nijmegen breakage syndrome (NBS) and prevalence of the major mutation, 657del5, in three Slav populations. *Eur J Hum Genet* 2000; **8**: 900–2.
11 Tauchi H, Matsuura S, Kobayashi J, Sakamoto S, Komatsu K. Nijmegen breakage syndrome gene, *NBS1*, and molecular links to factors for genome stability. *Oncogene* 2002; **21**: 8967–80.
12 Lee JH, Paull TT. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science* 2004; **304**: 93–6.
13 The International Nijmegen Breakage Syndrome Study Group. Nijmegen Breakage Syndrome. *Arch Dis Child* 2000; **82**: 400–6.
14 Seemanova E. An increased risk for malignant neoplasms in heterozygotes NBS syndrome of microcephaly, normal intelligence, growth retardation, remarkable facies, immunodeficiency and chromosomal instability. *Mutat Res* 1990; **238**: 321–4.
15 Tanzarella C, Antoccia A, Spadoni E *et al*. Chromosome instability and nibrin protein variants in NBS heterozygotes. *Eur J Hum Genet* 2003; **11**: 297–303.
16 Stumm M, Neubauer S, Keindorff S, Wegner RD, Wieacker P, Sauer R. High frequency of spontaneous translocations revealed by FISH in cells from patients with the cancer-prone syndromes ataxia-telangiectasia and Nijmegen breakage syndrome. *Cytogenet Cell Genet* 2001; **92**: 186–91.
17 Cheung V, Ewens W. Heterozygous carriers of Nijmegen breakage syndrome have a distinct gene expression phenotype. *Genome Res* 2006; **16**: 973–9.
18 Varon R, Reis A, Henze G, Van Einsiedel HG, Sperling K, Seeger K. Mutations in the Nijmegen breakage syndrome gene (*NBS1*) in childhood acute lymphoblastic leukemia (ALL). *Cancer Res* 2001; **61**: 3570–2.
19 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.
20 Cerosaletti KM, Morrison VA, Sabat DE, Willerford DM, Concannon P. Mutations and molecular variants of the *NBS1* gene in non-Hodgkin lymphoma. *Genes Chromosomes Cancer* 2002; **35**: 282–6.
21 Tessitore A, Biordi L, Flati V *et al*. New mutations and protein variants of *NBS1* are identified in cancer lines. *Genes Chromosomes Cancer* 2003; **36**: 198–204.
22 Dêbniak T, Górski 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.
23 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.
24 Cybulski C, Górski B, Dêbniak T *et al*. *NBS1* is a prostate cancer susceptibility gene. *Cancer Res* 2004; **64**: 1215–19.
25 Plisiecka-Hałasa J, Dansonka-Mieszkowska A, Rembiszewska A, Bidziński M, Steffen J, Kupryjańczyk J. Nijmegen breakage syndrome gene (*NBS1*) alternations and its protein (nibrin) expression in human ovarian tumors. *Ann Hum Genet* 2002; **66**: 353–9.
26 Górski B, Dêbniak T, Masojc B *et al*. Germline 657del5 mutation in the *NBS1* gene in breast cancer patients. *Int J Cancer* 2003; **106**: 379–81.
27 Steffen J, Nowakowska D, Niwinska A *et al*. Germline mutations 657del5 of the *NBS1* gene contribute significantly to the incidence of breast cancer in Central Poland. *Int J Cancer* 2006; **119**: 472–5.
28 Steffen J, Maneva G, Poplawska L, Varon R, Mioduszewska O, Sperling K. Increased risk of gastrointestinal lymphoma in carriers of the 657del5 NBS1 gene mutation. *Int J Cancer* 2006; **119**: 2970–3.
29 Chrzanowska KH, Piekutowska-Abramczuk D, Popowska E *et al*. Carrier frequency of mutation 657del5 in the NBS1 gene in a population of Polish pediatric patients with sporadic lymphoid malignancies. *Int J Cancer* 2006; **118**: 1269–74.
30 Warren S, Gates O. Multiple primary malignant tumors: survey of the literature and statistical study. *Am J Cancer* 1932; **16**: 1358.
31 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.
32 Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. *FASEB J* 1997; **11**: 68–76.
33 Kobayashi J, Tauchi H, Sakamoto S *et al*. NBS1 localizes to γ-H2AX: foci through interaction with the FHA/BRCT domain. *Curr Biol* 2002; **12**: 1846–51.
34 Hsu TC, Spitz MR, Schantz SP. Mutagen sensitivity: a biological marker of cancer susceptibility. *Cancer Epidemiol Biomarkers Prev* 1991; **1**: 83–9.
35 Taylor GM, O'Brien HP, Greaves MF *et al*. Mutations in the Nijmegen breakage syndrome gene (*NBS1*) in childhood acute lymphoblastic leukemia. *Cancer Res* 2001; **61**: 3570–2.
36 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**: 416–19.
37 Stanulla M, Stumm M, Dieckvoss BO *et al*. No evidence for a major role of heterozygous deletion 657del5 within the *NBS1* gene in the pathogenesis of non-Hodgkin's lymphoma of childhood and adolescence. *Br J Haematol* 2000; **109**: 117–20.
38 Rischewski J, Bismarck P, Kabisch H, Janka-Schaub G, Obser T, Schneppenheim R. The common deletion 657del5 in the Nibrin gene is not a major risk factor for B or T cell non-Hodgkin lymphoma in a pediatric population. *Leukemia* 2000; **14**: 1528–9.
39 Carlomagno F, Chang-Claude J, Dunning A, Ponder B. Determination of the frequency of the common 657Del5 Nijmegen breakage syndrome mutation in the German population: no association with risk of breast cancer. *Genes Chromosomes Cancer* 1999; **25**: 393–5.
40 Hama S, Matsuura S, Tauchi H *et al*. Absence of mutations in the *NBS1* gene in B-cell malignant lymphoma patients. *Anticancer Res* 2000; **20**: 1897–900.
41 Zienolddiny S, Campa D, Lind H *et al*. Polymorphisms of DNA repair genes and risk of non-small cell lung cancer. *Carcinogenesis* 2006; **27**: 560–7.
42 Cooper JS, Pajak TF, Rubin P *et al*. Second malignancies in patients who have head and neck cancer: incidence, effect on survival and implications based on the RTOG experience. *Int J Radiat Oncol Biol Phys* 1989; **17**: 449–56.
43 Ehlers JP, Harbour JW. NBS1 expression as a prognostic marker in uveal melanoma. *Clin Cancer Res* 2005; **11**: 1849–53.
44 Yang MH, Chiang WC, Chou TY *et al*. Increased NBS1 expression is a marker of aggressive head and neck cancer and overexpression of NBS1 contributes to transformation. *Clin Cancer Res* 2006; **12**: 507–15.