ORIGINAL ARTICLE

Increased Risk of Malignancies in Heterozygotes in Families of Patient with Nijmegen Breakage Syndrome (NBS)

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ABSTRACT

Background: The autosomal recessive chromosomal instability and hyperradiosensitivity Nijmegen breakage syndrome (NBS) in consequence of a mutation in the *NBS1* gene at 8q21 is associated with high occurrence of lymphoreticular malignancies due to defect of DNA repair (double strand breaks). In the Slavic population the majority of patients are homozygotes of the so-called "Slavic mutation" 657del5 in exon 6. Increased occurrence of malignant solid tumors (1) in families of NBS patients has been described even prior to the identification of the responsible gene, and the increased risk of malignancies in heterozygotes was thus hypothetical.

Methods and Results: The possibility of discerning mutation carriers in families from normal homozygotes enables verification of that hypothesis. Through molecular genetics investigations of grandparents and other relatives, we have been successful in determining the genotype in 79 of 112 grandparents in 28 families of our 39 patients. A single family had affected children in consequence of compound heterozygosity of the 657del5 and R215W mutations in the same exon of the *NBS1* gene. The proband's families were investigated genealogically and data on relatives were obtained over four generations. This data were repeatedly supplemented and objectively verified in church records and in healthcare documentation. Seven families have been followed up for 20 - 30 years, six families for 10 - 20 years, and 15 families for 1 - 10 years. Out of 28 families we were successful in examining the genotype of both grandparents in 16 families, there having been revealed one non-paternity; in six families only one of the grandparents has been examined; in five families we were not successful in examining any grandparent. Of 40 grandparents – normal homozygotes, a malignancy appeared in three (7.4 %), while among 39 heterozygotes of mutation 657del5 in the *NBS1* gene malignancies were documented in 15 (38,2 %) Mean age of NBS heerodygotes at manifestation of malignancy was 55.3 year (range 47 - 72 years), in the group of homozygotes it was 52.6 years (range 44 - 62 years). Nine grandparents died from malignancy prior to the discovery of the *NBS1* gene, and their genotype has been deduced genealogically in seven on the basis of the genotype in the wife and children, in two from preserved DNA. Out of that number, from three grandparents that had died from malignancies we were successful in obtaining neoplastic tissue for molecular genetics investigation, aimed at LOH or amplification of the *NBS1* gene. In another seven grandparents – heterozygotes, malignancies were manifest after determination of their genotype by DNA analysis, and consequently also from tumor tissue that has been obtained from three of them for molecular genetic investigation.

Conclusion: The age distribution and socio-economic status of both groups of grandparents did not differ, the sex ratio was slightly shifted towards females in the group of homozygotic grandparents (21 females and 16 males), and in the group of heterozygotes it was shifted towards males (20 males and 16 females). The sex ratio between heterozygotic grandparents with malignancies was likewise shifted towards the male gender (9 males and 4 females), in the group of homozygotic grandparents malignancy affected one male and two females. As verified in healthcare and church records, the occurrence of malignancies was significantly more frequent among grandparents heterozygotes for *NBS1* mutation than in healthy homozygotes.

Key words: autosomal recessive inheritance; DNA repair disorder, chromosomal instability; hyper-radiosensitivity, genealogic study; frequency of malignancies; NBS heterozygotes; normal homozygotes, 6574del5 mutation in NBS1 gene *Se.*

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INTRODUCTION

The chromosomal instability syndromes are associated with high cancer risk in homozygotes (3, 4) (30 - 40% in Fanconi anaemia (FA), Bloom's Syndrom (BS) and ataxia telangiectasia (AT), nearby 100 % in Nijmegen breakage syndrom (NBS) and Xeroderma pigmenosum (XP)). High risk of malignancy manifestation was considered and observed in their relatives. Some of them were confirmed as heterozygotes and others were presumable heterozygotes (2, 4, 5, 6, 7, 8, 9). Identification of mutation in FANC and XP genes is aggravated by genetic heterogeneity; there are more then 600 possible mutations of ATM gene. Bloom's Syndrom and Nijmegen breakage syndrome have low incidence in populations, except for Jewish (BS) and Slavic (NBS) population. However, the genetic heterogeneity is not known, and the number of mutations is low (4). The germ line mutation 657del5 is cause of the majority of NBS in the Slavic population, and thus identification of heterozygotes in families of patients and also in the population is not difficult (10). NBS is a disorder caused by mutations in a recently-identified gene called NBS1 (1), which encodes the production of nibrin. It is a protein whose absence is connected with cell and chromosomal hyperradiosensitivity (11, 12). DNA repair disorder predisposes all NBS homozygotes to the development of malignancies in young age. High occurrence of malignancies in NBS homozygotes raises a question of cancer risk for NBS heterozygotes

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(13). Swift referred to a slightly increased occurrence of solid tumors in AT heterozygotes (7, 8). Its cellular characteristics are identical with those of NBS, and that is why it was called "AT- variant" until the identification *NBS1* gene. Analysis of genealogic data of our NBS patients showed high occurrence of malignancies in their relatives (2). Occurrence of malignancies in two or more generations in direct line and manifestation of malignancies in the middle adult age matches the criteria of cancer families.

PATIENTS AND METHODS

We diagnosed 6 patients with NBS (two brothers and sisters and two singletous) for the first time in the former Czechoslovakia in 1979. Diagnosis was confirmed by detection of mutations in both their parents many years later. Up to date, we have diagnosed NBS in 24 children from 16 families born in the Czech Republic and in 15 children from 12 families born in the Slovak Republic in the years 1960-2004. Four-generation pedigrees were created in 28 proband families going back 1-30 years when diagnose of

Tab 1 History of grandparents of NBS patients before DNA analysis

Family	paternal grandparents	sex	age	malignity	last check-up	maternal grandparents	sex	age	malignity	last check-up
1	VA 1927	M	78	ne	2005	AB 1931–1999 iktus	M	68	ne	
	LA 1927–1999 iktus	F	72	ne		JB 1934	F	71	ne	2005
1	VA 1927	M	78	no	2005	AB 1931-1999 stroke	M	68	no	
	LA 1927-1999 stroke	F	72	no		JB 1934	F	71	no	2005
2	ZZ 1919-1967lymphoma	M	48	yes		OM 1926	M	79	no	2005
	AZK 1927	F	78	no	2005	EM 1930	F	75	no	2005
3	VD 1932	M	71	no	2003	JH 1934	M	69	no	2003
	ID 1935-1985 gyn ca	F	49	yes		OH 1945	F	58	no	2003
4	VČ 1921-1992 stroke	M	71	no		JM 1931	M	73	no	2004
	LČ 1923	F	81	no	2004	HM 1928	F	76	no	2004
5	MČ 1944	M	60	no	2004	FK 1931-2004 stroke	M	73	no	
	AČ 1945	F	59	no	2004	EK 1935	F	69	no	2004
6	IZ 1902-1990 age	M	88	no		ZO 1928-1996 ca prost	M	68	yes	
	HZ 1913	F	86	no	2000	VO 1929	F	74	no	2003
7	JP 1912-1983 lung ca	M	71	yes		JCh 1922	M	70	no	1992
	MP 1012-1985 stroke	F	63	no		HCh 1923	F	69	no	1992
8	MM 1896-1974 stroke	M	78	no		FS 1917 tumor cer 1979	M	84	yes	2002
	AM 1906-1972 gyn ca	F	66	yes		AS 1925-1979 stroke	F	54	no	
9	JD 1926-1996 stomach ca	M	69	yes		JF 1926-1995 stomach ca	M	68	yes	
	MD 1927	F	74	no	2001	LF 1928	F	73	no	2001
10	JF 1929	M	74	no	2003	MŽ 1943	M	60	no	2003
	IF 1927-2000 stomach ca	F	72	yes		AŽ 1945	F	58	no	2003
11	IP 1930-1987 lung ca	M	56	yes		GL 1936	M	63	no	1999
	IP 1933	F	66	no	1999	IL 1939-1983 gyn ca	F	44	yes	
12	SC 1925	M	75	no	2000	LR 1927	M	73	no	2000
	AC 1927	F	73	no	2000	PR 1930	F	70	no	2000
13	JH 1935	M	70	no	2005	LR 1947-1981 suicidium	M	34	no	
	LH 1940	F	65	no	2005	MR 1950	F	55	no	2005
14	JO 1940	M	60	no	2000	JP 1931	M	74	no	2005
	MO 1942	F	58	no	2000	AP 1932	F	73	no	2005
15	JS 1948	M	52	no	2000	JŠ 1942	M	58	no	2000
	OS 1948	F	52	no	2000	JŠ 1946	F	54	no	2000
16	AS 1936-1986 renal ca	M	49	yes		AB 1925	M	78	no	2003
	SS 1942	F	61	no	2003	MB 1937	F	66	no	2003
17	MG 1950 NH 2004	M	54	yes	2004	ŠB 1942	M	62	no	2004
	DG 1950	F	54	no	2004	VB 1952-2000 breast ca	F	47	yes	
18	MH 1932 stomach ca	M	66	yes	1998	FM 1930 ca recti	M	68	yes	1998
	TH 1930	F	68	no	1998	VM 1933	F	65	no	1998
19	JU 1951	M	54	no	2005	SN 1948-1992 stomach ca	M	44	yes	
	TU 1953	F	51	no	2005	JN 1952	F	53	no	2005
20	VG 1943	M	62	no	2005	VA 1938 ca colon 2003	M	67	yes	2005
	MG 1948	F	57	no	2005	IA 1945	F	60	no	2005
21	ŠF 1947-1998 stroke	M	51	no		JM 1945	M	58	no	2003
	BF 1949	F	54	no	2003	JM 1945	F	58	no	2003
22	VD 1940	M	65	no	2005	OK 1942	M	64	no	2004
	ED 1942	F	63	no	2005	MK 1945	F	59	no	2004
23	VŘ 1946	M	59	no	2005	??? Non-paternity				
	VŘ 1949	F	56	no	2005	JJ 1948	F	57	no	2005
24	JV 1915-1995 stroke	M	80	no		MA 1915-1981 ca ves uri	M	66	yes	
	VV 1916-1997	F	81	no		AA 1920-1997 colon ca	F	77	yes	
25	R215W mutation					JP 1952	M	53	no	2005
						AP 1948 basalioma 2001	F	57	yes	2005
26	KŠ 1927 colon ca 2004	M	78	yes	2005	JP 1945	M	60	no	2005
	JŠ 1931	F	74	no	2005	LP 1949	F	56	no	2005
27	JN 1937-2000 ling ca	M	63	yes		JP 1947	M	58	no	2005
	HN 1941 breast ca 2003	F	64	yes	2005	AP 1948	F	57	no	2005
28	AK 1910-1965 stroke	M	55	no		JN 1910-1973 stomach ca	M	62	yes	
	MK 1911-1990	F	79	no		MN 1914-1989 stroke	F	75	no	
total	54			13ca		55			12ca	

Legend to the table:
VA and AZK (2 x married, thus 2 names) and other are initials of grandparents (second column is paternal, seventh is maternal
F – female, M – male

Tab 2. Determination of genotype and occurrence of malignancies

Family	Heterozygote	sex	age	DNA	genea	Ca	Homozygote	sex	age	DNA	genea	Ca
1	LA 1927-1999	F	72	no	yes	no	VA 1927	M	78	yes	no	no
	JB 1934	F	71	yes	no	no	AB 1931-1999	M	67	yes	no	no
2	ZZ 1919-1967	M	48	no	yes	yes	AZK 1927	F	78	yes	no	no
	EM 1930	F	75	yes	no	no	OM 1926	M	79	yes	no	no
3	ID 1935-1985	F	49	no	yes	yes	VD 1932	M	71	yes	no	no
	JH 1934	M	69	yes	no	no	OH 1945	F	58	yes	no	no
4	LČ 1923	F	81	yes	no	no	VČ 1921-1992	M	70	no	yes	no
	JM 1931	M	73	yes	no	no	HM 1928	F	76	yes	no	no
5	FK 1931-2002	M	69	no	yes	no	EK 1935	F	69	yes	no	no
6	ZO 1928-1996	M	67	yes	yes	yes	VO 1929	F	73	yes	no	no
7												
8	AM 1906-1972	F	66	no	yes	yes	MM 1896-1974	M	78	no	yes	no
	FS 1917	M	84	yes	no	yes	AS 1925-1979	F	54	no	yes	no
9	JD 1926-1996	M	69	no	yes	yes	MD 1927	F	74	yes	no	no
	JF 1926-1995	M	68	no	yes	yes	LF 1928	F	73	yes	no	no
10	IF 1927-2000	F	72	yes	no	yes	JF 1929	M	75	yes	no	no
	MŽ 1943	M	61	yes	no	no	AŽ 1945	F	59	yes	no	no
11	IP 1930-1987	M	56	no	yes	yes	IP 1933	F	66	no	no	no
12												
13	LH 1940	M	65	yes	no	no	JH 1935	M	70	no	yes	no
	MR 1950	F	55	yes	no	no	LR 1947-1981	M	34	no	yes	no
14	JP 1931	M	74	yes	no	no	AP 1932	F	73	yes	no	no
15	JS 1948	F	52	yes	no	no	OS 1948	F	52	yes	no	no
	JŠ 1946	F	54	yes	no	no	JŠ 1942	M	58	yes	no	no
16	AS 1936-1986	M	49	no	yes	yes	SS 1942	F	61	yes	no	no
	AB 1925	M	78	yes	no	no	MB 1937	F	66	yes	no	no
17	MG 1950	F	54	yes	no	yes	DG 1950	F	54	yes	no	no
	VB 1952-2000	F	47	yes	no	yes	ŠB 1942	M	62	yes	no	no
18												
19	JU 1951	M	54	yes	no	no	TU 1953	F	52	yes	no	no
	JN 1952	F	53	yes	no	no	SN 1948-1992	M	44	no	yes	no
20	MG 1948	F	57	yes	no	no	VG 1943	M	62	yes	no	no
	VA 1938	M	67	yes	no	yes	IA 1945	F	60	yes	no	no
21	JM 1945	F	60	yes	no	no	JM 1945	M	60	yes	no	no
22	VD 1940	M	65	yes	no	no	ED 1942	F	63	yes	no	no
	MK 1945	F	59	yes	no	no	OK 1942	M	62	yes	no	no
23	VŘ 1946	M	59	yes	no	no	VŘ 1949	F	56	yes	no	no
	???						JJ 1948	F	57	yes	no	no
24												
25	JP 1952	M	53	yes	no	no	AP 1948	F	57	yes	no	yes
26	KŠ 1927	M	78	yes	no	yes	JŠ 1931	F	74	yes	no	no
	LP 1949	F	56	yes	no	no	JP 1945	M	60	yes	no	no
27	JN 1937-2000	M	63	no	yes	yes	HN 1941	F	64	yes	no	yes
	AP 1948	F	57	yes	no	no	JP 1947	M	58	yes	no	no
28												
Total	39	21:18		30	15	Ca	40	17:23		35	3	Ca

NBS was not confirmed, and therefore attention was not paid to malignancies occurrence. In addition, the NBS genotype of relatived treated for malignancy in time of examination was determined subsequently.

Genealogic study

Four-generation pedogrees with both parents and at least one of maternal and one of paternal grandparents was created by one person (ES). Data has been regularly augmented (at least once a year) during genetic care for the proband and his or her family and also during meetings of the Society for Chromosomal Instability Syndromes since 2000. We focused on occurrence of sterility, spontaneous abortions, deliveries of multiplets, congenital abnormalities, endogamy, and consanguinity. Grandparents were contacted and, providing they agreed, blood samples for molecular genetic investigation were obtained. The relatives were asked about working history and current occupation, contact with noxas including smoking and alcohol abuse, and also their personal history of diseases and X-ray examinations and current health status during examination. We obtained genealogic data and history data health directly from grandparents for practically in all families, though some of them had not experienced the discovery of NBS gene and the opportunity to determine genotype by DNA analysis. Three grandparents refused DNA examination; one of them could be identified as normal homozygote on the basis of the confirmation of mutation in his wife. The genotype was specified in 80 of 112 grandparents; one grandfather was excluded due to revealed non-paternity. We were able to examine genotype in 54 great-parents from parents and brothers and sisters of grandparents; 18 were heterozygotes and 36 were healthy homozygotes.

Molecular genetic investigation of Slavic mutation 657del5 in NBS1 gene at 8q21

Genomic DNA was prepared from non-coagulative peripheral blood using QIAamp Blood Kit and 657del5 mutation was determined as described earlier (14).

Tab. 3. NBS patients and malignancies

Family	patient	ethnicity	sex	birth	malignancy	malig	age death	cause of death
28	MK	C	F	1960	0		1961	enterocolitis
28	AK	C	M	1961	0		1962	pneumonia
8	DM	S	F	1969	lymphogranuloma	9	1979	lymphogranuloma
7	JP	C	M	1970	ALL	9	1980	ALL
9	MD	C	F	1971	NHL	18	1993	pneumonia
23	SV	S	F	1973	0			
11	ZZ	S	F	1973	gonadoblastoma	18	1994	gonadoblastoma
4	JC	C	F	1975	NHL, NHL	26, 29	2004	syncope
9	DD	C	M	1977	lymphosarcoma	1,5	1979	lymphosarcoma
7	EP	C	F	1978	ALL	1	1979	ALL
4	RC	C	F	1979	Ewing sarcoma	13	1995	renal insufficiency
6	RZ	C	F	1979	malignant meningeoma		12	
1	PA	C	M	1980	ALL, Hodgkin lymphoma		13, 17	
2	LZ	C	F	1980	0		1980	pneumonia
2	JZ	C	M	1981	NHL	8	1991	NHL
18	JP	G-S	F	1983	medulloblastoma	6	1990	medulloblastoma
12	MH	S	M	1984	0			
2	N	C	M	1984	0		prenatal diagnose	
16	JS	S	F	1985	NHL	16	respiratory insufficiency	
3	LD	G-C	F	1986	NHL	6	1996	
5	MC	C	M	1988	NHL	6		
12	LH	S	M	1989	ALL	5	2003	ALL
14	MO	S	M	1989	NHL	10	2005	NHL
10	MF	S	F	1989	0			
13	JH	C	M	1991	NHL	6	1998	NHL
15	JS	C	M	1992	ALL	6	1999	ALL
2	N	C	F	1992		0	prenatal diagnose	
29	BP	C	F	1993		0		
21	AD	S	F	1996		0		
17	MG	S	F	1999		0		congenital hydrocephalus
22	MF	S	M	2000		0	2000	
19	DU	C	M	2001		0		
21	MD	S	M	2001	rhabdomyosarcoma	0,5		
20	TG	C	M	2002		0		
22	N	S	F	2002		0	prenatal diagnose	
26	MS	S	M	2002		0		
24	NR	C	F	2002		0		
25	JR	C	M	2003		0		
25	PR	C	M	2003		0		
27	MN	C	F	2004	0			
29 families		17 C 10 S 2 G	19M 21 F		20 + 2 variance 0,5–29	mean age 9 years		

MK, AK and other up to MN are initials of patients
C – Czech ethnic, S – Slovak ethnic, GC – Romany Czech ethnic, GS – Romany Slovak ethnic F – female, M – male, NHL – Non-Hodgkin's Lymphoma, ALL – Acute Lymphocytic Leukaemia

RESULTS

Genealogy

Mean age of 111 grandparents at last contact or at death was 58 years (variance 44 - 58 years). 25 grandparents suffered from malignance (16 M, 8 F); it was not possible to determine genotype genealogically in 7 (6 M a 1 F) of them. **See Tab. 1 and Tab. 2.**

Genotypes of 18 grandparents with malignancy were determined genealogically in 8 cases and by DNA analysis in 9 cases. 657del5 mutation was detected in 10 of them (5 were detected by DNA analysis and 8 genealogically). It was possible to examine 54 brothers and sisters and parents of grandparents by DNA analysis. 18 were heterozygotes of 657del5 mutation and the mutation was excluded in 36 cases.

We succeeded in determining genotype in 79 grandparents; in 65 of them by direct detection of mutation 657del5 and in 14 on the basis of examination of relatives, husband/wife and children. A malignant tumor was detected in 15 of 39 heterozygotes, from 5 of them histological samples of malignant tumour were

Tab. 4. Occurrence of malignancies in grandparents and great-grandparents after genotype determination

grandparents	NBS heterozygotes	homozygotes
number of persons	39	40
M:F	21:18	18:22
Mean age	61,1 years	63,9 years
genotype DNA	30	35
genotype geneal	9	5
number of malignancies	15	3
M:F	11:04	1:02
Frequency of malignancies	38,50 %	7,40 %
Mean age malign	59,3 years	52,6 years
Significant differentiation P > 0,01		
Great-grandparents and granduncles/grandaunts according to genotype		
NBS heterozygotes		
Number of persons	18.	36.
M:F	10:8	24:12
DNA genotype	18	36
Mean age	63,9 years	73,2 years
Number of malignancies	5	2
M:F	2:3	0:2
Mean age malign	66,2 years	45,0 years
Frequency of malignancies	27,70 %	5,50 %
Significant differentiation P > 0,01		

obtained, either for tumor verification, molecular genetic investigation aimed at LOH, or amplification of allele of germinal mutation. The malignancies were verified according to the death certificate in another 6 grandparents, and only in 3 grandparents were the data from history and further unverifiable. The absence of 657del5 mutation among 40 grandparents was verified by DNA analysis in 35 of them and in 5 of them on the basis of genealogy (examination of husband/wife). One grandfather was heterozygote of R215W mutation. A malignant tumor was detected in 3 of them, in one case from the death certificate and in 2 from health-care documentation.. It was possible to prove malignancy in 38.5 % in group of heterozygotes while only in 7.5 % in group of non-heterozygotes. The difference is statistically significant (P<0.01 using Chi-square test). Malignancy as a cause of the death was present in 7 cases (2 x lung carcinoma, 2 x stomach carcinoma, 2 x colorectal carcinoma and 1 carcinoma vesicae urinal) of 32 grandparents whose genotype determination failed even by genealogically.

The mean age of all grandparents was 60.5 at malignancy manifestation or death. It was 55.3 years in the group of heterozygotes and 52.6 in the group of homozygotes. The difference is not significant.

Five cases of malignancy (1 x lymphoma, 1 x multiple myeloma, 1 x gynaecological malignancy, 1 x pancreatic and 1 x Merkel carcinoma and subsequently breast cancer after radiotherapy of the original malignancy after 6 years) were detected in the group of 18 heterozygotes out of 54 relatives. Only 2 malignancies were detected in the group of 36 homozygotes (1 x colon, 1 x ovarium). The difference is significant (P< 0.01).

Tumors in heterozygotes of 657del5 mutation occurred without predisposition. These comprised stomach lymphoma, two gynaecological carcinoma, three stomach carcinoma (2M, 1F), three colon-carcinoma (3M), one brain tumor (M), one breast cancer, one prostatic carcinoma and two lung carcinoma (2M) in verified malignancies of grandparents-heterozygotes.

One stomach carcinoma, one breast cancer and one basalioma in a woman who worked as an X-ray technician for 40 years occurred in group of non-heterozygotes.

The results of the study supports the hypothesis that heterozygosis of NBS1 mutation is connected with 38.5% risk of manifestation of malignant tumor by the age of 75. It is significantly higher probability than in grandparents – nonheterozygotes (7.5 %) and also than in general population (22,671 tumors among 5,013,000 males and 21,820 tumors among 5,310,000 females were reported in 1993 (15)). We also found a significant difference in malignancy occurrence between heterozygote and homozygote in the group of great-grandparents and grand uncles/grand aunts of NBS patients.

DISCUSSION

Occurrence of malignant tumours among the population is common, but only in 10 % could genetic etiology (germinal mutation of oncogene or tumor suppressor gene) be identified. The occurrence of malignancies in our cohort of grandparents and great-grandparents of patients with syndrome of chromosomal instability - Nijmegen breakage syndrom (NBS) (whose age distribution, social environment and mutagens exposition was comparable) was significantly higher in the group of NBS heterozygotes as compared with the group of homozygotes of normal alleles. The results of the study support the hypothesis that heterozygosity of 657del5 mutation is connected with significantly increased cancer risk by the age of 70 as compared with non-heterozygotes *NBS1* and also as compared with the general population (15). We conclude that we have succeeded in proving that heterozygotes of Slavic 657del5 mutation in NBS1 gene also have significantly increased risk of malignancy – universally of solid tumors – without special predisposition.

Increased risk of malignancies in heterozygotes of chromosomal instability syndromes was repeatedly considered and verified mainly in relatives with mutation in the ATM gene (7, 8, 9) as a result of deficient DNA reparation (11, 13). However, the identification of heterozygotes in families of patients with affections with genetic or gene heterogeneity is difficult; to date it is actually impossible in the general population. The etiology of NBS is homogenous in the Slavic population, which is why the identification in families of patients and in population of heterozygotes is trouble-free.

The frequency of heterozygotes of 657del5 mutation found in Slovak population was 0.5 – 1 % (16). Their high risk of manifestation of malignant tumors in middle age can represent a significant proportion of oncology patients. Hyperradiosensitivity of 657del5 heterozygotes was repeatedly demonstrated (11, 12, 13) by increase in chromosomal and chromatides breakage after *in vitro* exposition to ionising radiation. Hyperradiosensitivity of heterozygotes should be respected during tumor therapy and in prevention of secondary malignancies, and especially in prevention of primary tumors. High occurrence of malignancies in cohort of grandparents of our heterozygotes could be the result of genotype and high exposition of our population to ionisation during the years 1952 – 1992, when X-ray examination was widely used for the detection of hip dysplasia in infants and for screening of tuberculosis in children and adults. Moreover, our geographic location near uranium mines of Jáchymov, Příbram and Rožínky can contribute to higher exposure of our population to ionising radiation as compared with other Slavic populations. Combination of genotype and external factors during tumor manifestation in heterozygotes of 657del5 mutation offers efficient prevention of increased risk of malignancies in heterozygotes. Improvement in phenotypical symptoms could be expected from protection against ionisation and from sufficient supplementation with antioxidants.

List of used abbrevations

AT – Ataxia telangiectasia
BS – Bloom Syndrom
FA – Fanconi anaemia
NBS – Nijmegen breakage syndrome
XP – Xeroderma pigmenosum

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