Nijmegen breakage syndrome (NBS) is a deficient DNA repair (inherited in an autosomal recessive manner) with hyperradiosensitivity and a high occurrence of lymphoreticular malignancies, which is detected mostly in Slavic populations (1–3). All NBS patients with Slavic ancestors were homozygotes or compound heterozygotes of 5bp deletion in exon 6 (mutation 657del5) (1,2) to date.

The frequency of heterozygotes of mutation 657del5 found in our population is 0.5 – 1 percent (4), and the frequency of heterozygotes of R215W detected in our country is 1:234 (5). Genealogy of patients with NBS provided evidence of increased occurrence of malignancies in heterozygotes in middle or older age (6, 7) and both heterozygotes of another chromosomal instability and hyperradiosensitivity syndrome - Louis- Bar’s ataxia telangiectasia. Nibrin, a protein product of NBS1 gene, is a part of the MRE11/RAD50 complex similar to 350-kDA protein product of ATM gene, which is responsible for ataxia telangiectasia (2). This complex is responsible for DNA repairs (double strand breaks-DSBs), and that is why carriers of mutations in NBS1 and ATM genes have insufficient reparation of chromosomal spontaneous breaks, but mainly of aberrations, induced by radiation.

Protection of heterozygotes of tumor suppressor gene NBS1 against ionising radiation is viewed as an effective prevention of malignancy development. Detection of heterozygotes among their children and other relatives of patients with malignancy that are carriers of mutation offers possibility of efficient prevention against malignancies by avoidance from ionization mutagens.

The aim of this study is to find out the occurrence of heterozygotes of mutations 657del5 and R215W among adult patients with malignancies in comparison with occurrence of heterozygotes among newborns and individuals older than 70 years (10, 11).
We collected dry blood spots on screening papers from 706 patients treated for different types of malignancies primarily at the departments of Charles University 2nd Faculty of Medicine and Faculty Hospital Motol (Prague) during the years 2000-2004. Table 1 shows the number of assessed patients with particular tumor types.

Most patients were older than 50 years, but it is not possible to establish either mean age or median because of 228 anonymous specimens of patients with NHL. The sex ratio was not significantly shifted as compared with 1:1. It cannot be specified with respect to anonymous specimens of patients with NHL.

Detection of mutation 657del5 was performed in Prague and detection of mutation R215W in Berlin. Methods used did not differ between the two locations. A piece size 2 mm in diameter was separated from each blood spot and used for mutation analysis by means of PCR-SSCP (polymerase chain reaction-single strand conformation polymorphism) in PCR reaction with primers marked with fluorescence specific for exon 6 of \textit{NBS1} gene, as has repeatedly been described (7, 11). All specimens where aberrant shift was detected were subsequently sequenced, and in this way the second mutation R215W was proved.

### RESULTS

The occurrence of \textit{NBS1} heterozygotes of mutations 657del5 and R215W in exon 6 in particular groups of patients with malignancies is showed in Table 2.

The expected occurrence of heterozygotes would be according to the population data (1:128–165 among newborns) in cohort of 706 patients with malignancies 4.2-5.4 persons. The difference between occurrence of 5 heterozygotes of mutation 657del5 among 706 adult patients with malignancy and the occurrence of heterozygotes in population of newborns is not significant (chi-square –test); it is the same if we divide the cohort according to tumor types. Frequency of heterozygotes of mutation 657del5 in \textit{NBS1} gene increases in comparison with the expected count according to the population data in groups of patients with colorectal tumor on the margin of significance and non-significantly increased in the group of patients with breast cancer and skin tumors.

However, if we compare occurrence of \textit{NBS} heterozygotes among patients with malignancy and population of individuals at the age of 70 and older, (1:423), the difference would be significant, also in comparison with the occurrence in the whole cohort of adult patients older than 50 years.

### DISCUSSION

Increased frequency of malignant tumors among relatives of patients with \textit{NBS} and AT which were carriers of germinal mutations in \textit{v ATM} and \textit{NBS1} genes has repeatedly been described (6, 8, 9, 12, 13).

Frequency of \textit{NBS} heterozygotes in populations that are decreasing with the increasing age could show loss of \textit{NBS} heterozygotes due to the death caused by malignancy (10, 11). This finding and easy identification of mutation with predominant occurrence in Slavic population (2) in \textit{NBS} gene led to study of heterozygotes frequency among patients with malignancy and also in tumor tissues (14) of heterozygotes of germinal mutations. Steffen et al. found significantly more mutations 657del5 and R215W than would correspond with occurrence in population in the group of more than 1000 oncology patients in the year 2004 (13). Significantly increased occurrence was detected mainly in groups of patients with melanoma, breast carcino-
ma, colorectal carcinoma and NHL. NBS1 gene was confirmed as tumor suppressor gene (14, 15) by verification of loss of heterozygosity (LOH) or by detection of gene amplification in tumor tissue. The results of our smaller cohort of adult patients with malignancy correspond with these results as far as germline mutation 657del5 is concerned, with the exception of patients with NHL that was detected by Souček et al (16). We found only one heterozygote of mutation R215W among 483 assessed patients with malignity, although the frequency of this mutation found in the population of newborns was 1:253 and the expected number would be 2. The occurrence of this mutation in a cohort of Polish oncology patients was significantly increased (13). The difference could be explained by the small number of patients in the cohort, particularly after its division according to particular tumor types.

The occurrence of heterozygotes of both mutations in NBS1 gene – 657del5 and R215W – is 1 percent in Slavic population (2, 4). Also the medium-increased risk of malignancies in these hyperradiosensitive individuals can thereafter comprise development of many new malignancies every per year.

DNA hyperradiosensitivity of carriers of mutation in NBS1 gene offers the possibility of efficient prevention of malignancies or delaying of manifestation to older age by protection against ionizing radiation, control of levels of chromosomal instability and by sufficient intake of antioxidants. Detection of mutation in NBS1 gene in adult oncology patients plays a less important role in prevention of secondary malignancies than in treated child patients. However, it could be an important preventive measure for their children and other involved relatives and for the improvement of both own clinical prognosis and genetic prognostics of their reproduction.

### Abbreviations

- AT – ataxia telangiectasia
- LOH – loss of heterozygosity
- NBS – Nijmegen breakage syndrome
- NHL – non-Hodgkin’s lymphoma
- PCR-SSCP – polymerase chain reaction-single strand conformation polymorphism

### REFERENCES


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