

Nijmegen Breakage Syndrome in 13% of Age-Matched Czech Children With Primary Microcephaly

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The Nijmegen breakage syndrome is a rare autosomal recessive chromosomal instability disorder characterized by early growth retardation, congenital microcephaly, immunodeficiency, borderline mental development, and a high tendency to lymphoreticular malignancies. Most Nijmegen breakage syndrome patients are of Slavonic origin, and all of them known so far carry a founder homozygous 5 nucleotide deletion in the NBS1 gene. Microcephaly was present in 100% of Nijmegen breakage syndrome patients in a recent large international cooperative study. The frequency of Nijmegen breakage syndrome among children with primary microcephaly was not known. Early correct diagnosis of the syndrome is crucial for appropriate preventive care and therapy. We tested 67 Czech patients of different ages with simple microcephaly for the presence of the most common mutation in the NBS1 gene. Three new Nijmegen breakage syndrome cases were detected in this cohort, representing 4.5% of the cohort. All these newly diagnosed Nijmegen breakage syndrome patients were younger than 10 months at the time of diagnosis. They were all born within a 2.5-year period. Twenty-three of the 67 children in the cohort were born within this 2.5-year period, representing a 13% incidence of Nijmegen breakage syndrome. Frequency of Nijmegen breakage syndrome heterozygotes among infants in the Czech Republic is 1:130-158 and the birth rate is 90,000 per year, therefore in the time span of 2.5 years, three new Nijmegen breakage syndrome homozygotes are expected to be born. Therefore we assume that by DNA testing of Czech primary microcephalic children it is possible to detect all Nijmegen breakage syndrome patients to be expected. The age at correct diagnosis was lowered from 7.1 years at the time before DNA

testing, to well under 1 year of age. All new Nijmegen breakage syndrome patients could receive appropriate preventive care, which should significantly improve their life expectancy and prognosis. © 2004 by Elsevier Inc. All rights reserved.

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Introduction

In 1981 Weemaes et al. described two brothers with congenital microcephaly, immunodeficiency, recurrent respiratory infections, and chromosomal breakage. This new disorder is called Nijmegen breakage syndrome (NBS) [1]. In 1985 Seemanová et al. described a high risk for lymphoreticular malignancy in nine NBS patients [2]. Until 1998 the diagnosis of NBS was possible only on clinical ground and at the cytogenetic level. In 1998 Varon et al. demonstrated that nearly all NBS patients are homozygous for the same founder mutation—deletion of 5 bp (657del5) in the NBS1 gene, encoding the protein nibrin [3]. Most NBS patients are of Slavonic origin and therefore, this frameshift mutation came to be called “Slavonic mutation”.

Based on the heterozygous frequency of the Slavonic mutation among infants (1:130 to 1:158) [4,5], the frequency of homozygotes should be 1:67,600 to 1:99,900. The birth rate in the Czech Republic is 90,000 liveborns per year or 225,000 during 2.5 years, therefore statistically three new NBS homozygotes—NBS patients are expected to be born within a 2.5-year period.

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Early, etiologically correct diagnosis is crucial for appropriate preventive care with consequent protection against ionizing irradiation and with reduced chemotherapy of malignancy [6]. Congenital microcephaly is an obligate sign of NBS, which was present in all 55 patients in a large cooperative clinical NBS study [7]. Microcephaly in NBS is progressive and severe; no correlation was detected between head circumference at birth and mental development [4,7].

Microcephaly is defined as an occipitofrontal circumference under the third percentile or below 2 standard deviations [8]. Microcephaly can be secondary, caused by different exogenous insults such as prenatal infection, trauma or due to a neurodevelopmental disorder or as a part of some known syndrome. Microcephalia vera or pure microcephaly without any obvious origin and without any additional malformations is called primary microcephaly [8]. There were no data about the frequency of NBS among children with primary microcephaly before the start of our study. To estimate the frequency of NBS among Czech children with primary microcephaly and to attempt to detect all newly born NBS homozygotes, we performed DNA testing for the presence of 657del5 mutations among, in total, 67 primary microcephalic children.

Patients and Methods

Patients with simple microcephaly with an occipitofrontal circumference under the third percentile without any associated malformations and without any clear etiology of microcephaly were referred for DNA testing from different pediatric, neuropediatric, or clinical genetic departments across the Czech Republic. The assessment of the occipitofrontal circumference and microcephaly was made by the referring physicians, and not in every case was it reassessed by the authors. The testing period was from November 1999 to May 2002.

Sixty-seven patients (37 male, 30 female) referred for primary microcephaly were tested for the presence of the 657del5 mutation in the NBS1 gene. The youngest patient was 6 months old and the oldest 22 years. The average age in the group was 6.6 years (6.5 years among males and 6.7 years among females). From the 67 patients with primary microcephaly of different ages, 6 were born in the year 2001, 8 in 2000, 9 in 1999, 8 in 1998, 5 in 1997, 6 in 1996 and 2 in 1995. The remaining 23 patients were born before 1995.

DNA was isolated from peripheral blood; alternatively dried blood spots were also used as a direct template for the polymerase chain reaction. Two different methods for detecting the Slavonic mutation were used alternatively during this study. In one method, a pair of intronic primers flanking exon 6 of the NBS1 gene (NBS Ex6 F: 5'; CAG-ATA-ACT-CCG-TTT-ACA-A, NBS Ex6 R: 5'; ATG-AAT-AGG-CCA-GTG-ATC-ACA-G) were used to amplify the entire exon 6 containing the region of the Slavonic mutation. The forward primer was labeled with fluorescent dye HEX for the subsequent analysis of the fragment length and number on an automated capillary electrophoresis Genetic Analyzer ABI310 (ABI, Foster City, CA) using the Gene Scan software, POP 4 polymer, and Tamra 500 internal size standard (ABI, Foster City, CA). The wild-type fragment is 404 bp; in the presence of 657del5 mutation in the heterozygous state, two fragments are detected (399 bp and 404 bp) and the 657del5 homozygosity results in the presence of only one shorter 399 bp product (Fig 1). Alternatively, we used a simpler method without using the ABI310 analyzer, whereby a shorter fragment of 60 bp flanking the region of 657del5 mutation in exon 6 of NBS 1 gene is amplified

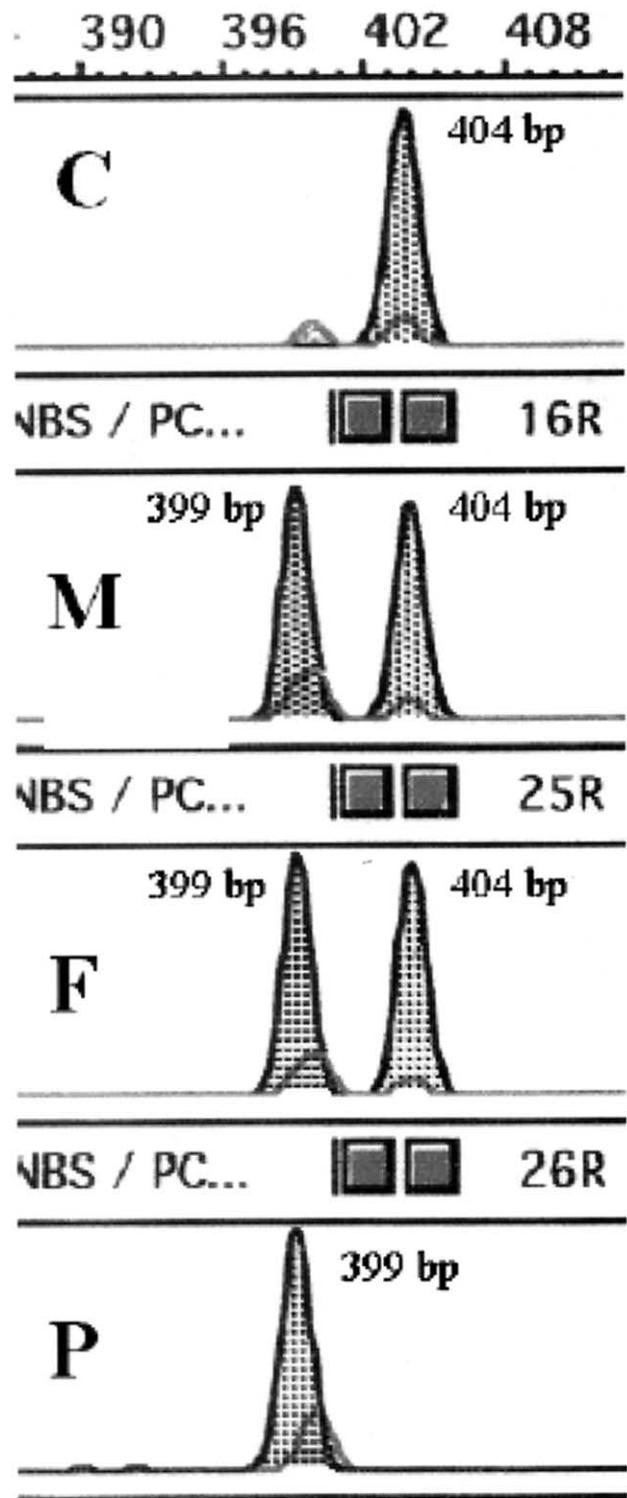


Figure 1. Results from our detection method using ABI310 and Gene Scan software. In the first line (C for control) is the result from a normal homozygous control; note only one longer 404 bp product. In the second and third lanes are parents of an NBS child (M for mother and F for father), heterozygous for the 657del5 mutation; note two peaks, one with deletion 399 bp and the second normal 404 bp. In the lowest line (P for patient) is the result from a NBS patient, homozygous for the 657del5 mutation; note only one, shorter 399 bp product.

using the primers 657del5 F (5'; AAT-GTT-GAT-CTG-TCA-GGA-CG) and 657del5 R (5'; TAT-AAA-TGT-TTT-CCC-TTT-GAA-GA). The resulting product was then electrophoresed in 3% high-resolution agarose

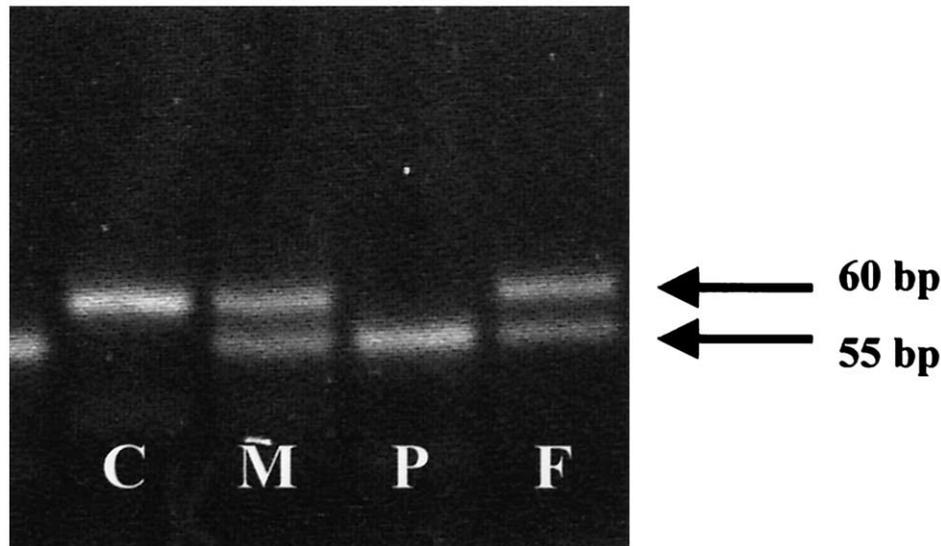


Figure 2. Results from our detection method using high-resolution agarose. Lanes M (mother) and F (father), NBS heterozygotes—patient's parents; lane P (patient)—NBS homozygote; lane C (control) normal homozygous control.

gel Meta Phor (Promega, Madison, WI). The wild-type allele manifests one 60 bp fragment, a 657del5 homozygote has one shorter 55 bp fragment, and a 657del5 heterozygous carrier displays two fragments of 55 and 60 bp (Fig 2).

Polymerase chain reaction conditions were the same in both reactions: initial denaturation 95°C for 5 minutes followed by 30 or 35 cycles, respectively of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 2.5 minutes.

Results

Sixty-seven Czech children of different ages referred with primary microcephaly, without any clear etiology were tested for the presence of the most common mutation in the NBS gene. Three new NBS cases, homozygous for the 657del5 mutation, were detected in this group. The resulting frequency of NBS homozygotes among patients of different ages with primary microcephaly in this study was 4.5%. Within the period of the 2.5 years that all the newly detected NBS patients were born, there have also been born 23 children of the 67 from our cohort with primary microcephaly. Thus in the age-matched cohort with primary and congenital or early-onset microcephaly in the Czech Republic, NBS represented 13%. These three cases are equal to all new NBS cases expected to be born in the Czech Republic during a period of 2.5 years, i.e., the interval in which they have been born. DNA testing of only congenitally and primary microcephalic children could be sufficient for early detection of all, or nearly all,

new NBS cases in the entire population without any need of a whole-population screening.

Clinical data from all three newly diagnosed NBS patients are summarized in Table 1. All children were microcephalic at birth, and the microcephaly remained well under the third percentile even when growth of the child was at the third percentile. The psychomotoric development has been normal in all three children thus far. All three NBS infants manifested early hyperactivity and a friendly phenotype (Figs 3-5). None of them have developed any malignancy or immunodeficiency complications. As a result of good cooperation among pediatricians, neuropediatricians, and geneticists, the age of correct diagnosis of NBS has decreased from 7.1 years at the time before DNA testing [4] to an age well under 1 year, after introduction of DNA testing.

Discussion

NBS was clearly underdiagnosed in the past, with only 25 detected cases in the period 1969 through 1992 during which 5.2 million infants were born in Czechoslovakia at the known heterozygous frequency of 1:130 to 1:154 among infants [2,4].

By DNA testing of primary microcephalic children, mainly those who were already microcephalic at birth or infancy, we were able to detect early all expected NBS

Table 1. Principal clinical data from all three new NBS children

Patient	Sex	Birth Date	Gestation Week at Birth	Birth Head Circumf. (cm)	Birth Weight (gm)	Birth Length (cm)	Age at Diagnosis (mo)
M	F	26 Feb. 99	40	29	2500	46	7
D	M	1 Sept. 00	41	32.5	3100	48	8
T	M	1 Aug. 01	41	30	2700	48	7

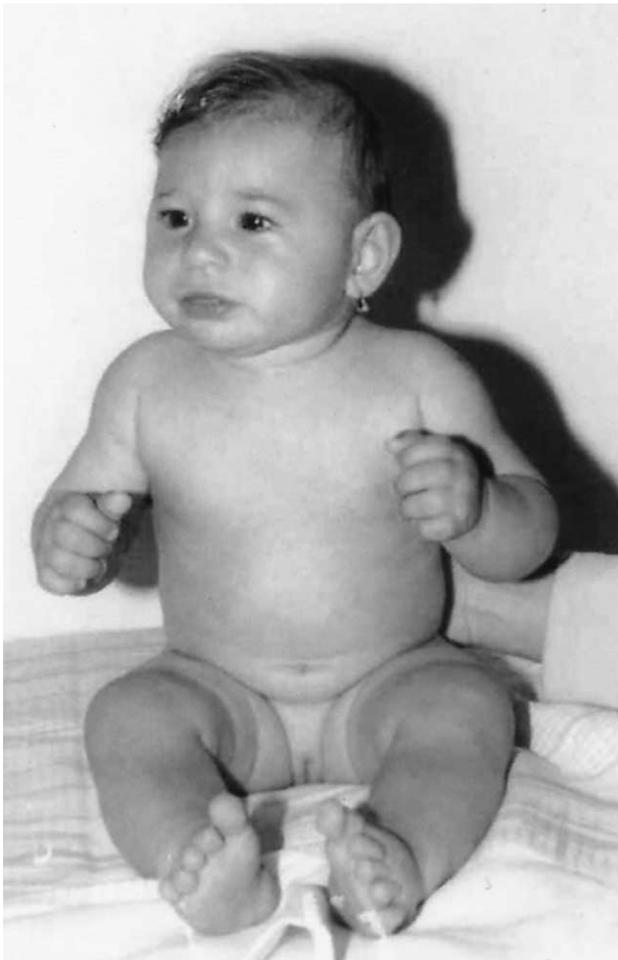


Figure 3. Patient M (girl) at the age of 7 months when the diagnosis of NBS was confirmed by DNA testing and the presence of homozygosity for the Slavonic mutation was detected. Head circumference at the time was 35.5 cm (well under the third percentile). Note an eutrophic habitus, normal motoric development, active facial expression, and typical facial characteristics of NBS—prominent midface, oblique eyelids, retromandibula, large ears.

children newly born in the Czech Republic in the period from February 1999 through August 2001. The incidence of NBS of 13% that we have observed in our cohort of children with primary and early-onset microcephaly clearly supports the indication of DNA testing for the 657del15 mutation in the NBS1 gene in all infants suffering congenital or early-onset microcephaly without any clear etiology in the future. Our results indicate that the screening of only microcephalic infants with good cooperation among pediatricians, neuropediatricians, and geneticists can be sufficient to detect all NBS homozygotes expected to be newly born in the population without the need for whole-population screening.

Early detection of NBS is crucial for appropriate preventive care and treatment of affected patients and should improve their life prognosis. It is also important for effective prenatal diagnosis in the family. To a physician with NBS experience, the NBS phenotype is easy to recognize by typical microcephaly, birdlike face, and

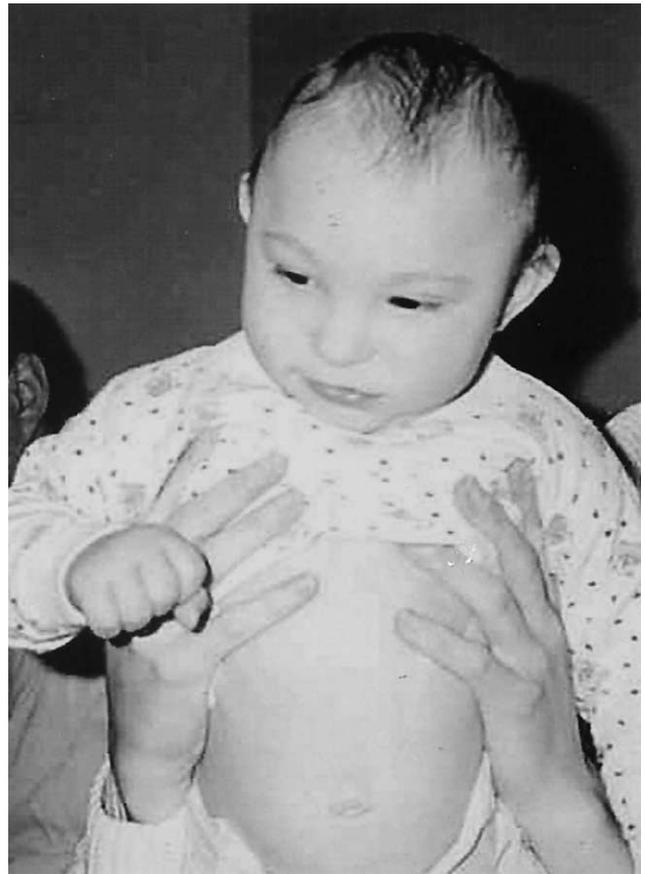


Figure 4. Patient D (male) at the age of 8 months when the diagnosis of NBS was confirmed by DNA testing. Note eutrophy, large ears, prominent midface, receding mandibula, and active overall facial expression.

active behavior; however, there are still cases with severe microcephaly correctly diagnosed late by the presence of some more severe complications or malignancy, as recently reported from Poland [9].

All the newly and early detected NBS patients in this study were able to receive sufficiently early appropriate preventive care through complete avoidance of exposure to x-rays and protection from other mutagens. Later in life, in case any of them develops highly probable malignancy, a reduced dosage of chemotherapy avoiding radiomimetic cytostatica will be applied. The treatment of malignancy is only successful if it respects the hyperradiosensitivity of the patients. According to our experience, treatment with substantially reduced doses of chemotherapy is successful and the majority of earlier diagnosed NBS patients if treated this way survive, some of them even more than one malignancy. The consequent avoidance of radiotherapy also plays a positive role in the prevention of secondary tumors [6].

Until 1997, NBS was also named ataxia-telangiectasia variant 1 and 2 (AT-V1, AT-V2), because at the cellular level there were strong cellular and chromosomal similarities [10]. At the clinical level, the differential diagnosis of NBS and ataxia-telangiectasia is unproblematic, because



Figure 5. Patient T (male) at the age of 7 months when the diagnosis of NBS was confirmed by DNA testing. His head circumference at that time was 36 cm. Note small receding chin, large ears, prominent midface, and oblique eyelids.

hypoplasia of cerebellar vermis (but no microcephaly) leads to ataxia since infancy in young children with ataxia–telangiectasia and serum alpha fetoprotein is highly increased in 95% of ataxia–telangiectasia patients [11], which are not the features in NBS [4,7]. The ATM gene, mutated in ataxia–telangiectasia, is located on chromosome 11q22-23; it is large with more than 300 different mutations distributed within 66 exons of the gene without any common, predominant mutation as in NBS, which greatly limits the utility of DNA testing as a diagnostic tool in ataxia–telangiectasia [12].

The NBS1 gene is located on chromosome 8q21, and only seven different mutations were reported in this gene worldwide, with high predominance of the founder mutation 657del5 in the Slavonic population [3]. Protein products of both the NBS1 and AT genes—nibrin and ATM protein—participate in the formation of the protein complex hMRE11/RAD50 involved in the repair of DNA double-strand breaks [13]; therefore, both diseases manifest chromosomal instability, hyperradiosensitivity, and

increased risk for developing lymphoreticular malignancies. Prevalence of ataxia–telangiectasia seems to be rather higher in the Slavonic population (1:40,000 to 1:100,000) than that of NBS in the Slavonic population (1:60,000 to 1:120,000) [14].

Microcephaly is a typical component of many other syndromes and conditions. Among the most common pediatric disorders in which microcephaly is present are Rett syndrome [15], Angelman syndrome [16], or in some cases also fragile X syndrome [17]. These disorders are clinically characterized extremely well, but the microcephaly develops later in life and is not present at birth as in infants with NBS [4,7].

Children with primary microcephaly, which is an easily recognizable sign present already at birth, should be tested for the presence of 657del5 mutation in the NBS1 gene. Such a procedure can at least in Slavonic patients detect early all newly born NBS children, who can profit from the appropriate preventive care and therapy.

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