Electronic letter

J Med Genet 2001;**38** (http://jmedgenet.com/cgi/content/full/38/1/*e*3)

Atypical clinical picture of the Nijmegen breakage syndrome associated with developmental abnormalities of the brain

EDITOR—Nijmegen breakage syndrome (NBS) (OMIM 251260) is a rare autosomal recessive condition. The major manifestations include microcephaly, a distinct facial appearance, growth retardation, recurring infections owing to combined immunodeficiency, spontaneous chromosomal instability (with characteristic rearrangements of chromosomes 7 and 14), hypersensitivity to ionising radiation, and a very high predisposition to lymphoid malignancy. The immunological, cytogenetic, and cell biological findings are very similar to those in ataxia telangiectasia (AT); however, NBS patients lack the neurocutaneous manifestations of AT, as well as a raised serum α-fetoprotein (AFP).[1–3] Recently, the gene mutated in NBS has been cloned. The *NBS1* gene is located on chromosome 8q21 and encodes a protein called nibrin, a member of the hRad50/hMre11 protein complex involved in DNA double strand break processing.[4 5] NBS is quite a rare disease with about 80 patients ascertained world wide, including 50 Polish. The vast majority of NBS patients are of Slavic origin and carry a common founder mutation 657del5 in exon 6.[4]

We report on a Polish patient with Nijmegen breakage syndrome presenting an atypical clinical picture with the absence of microcephaly and the presence of a congenital heart defect and preaxial polydactyly. To the best of our knowledge, no NBS patient without microcephaly or with congenital cardiac disease has ever been reported and preaxial polydactyly has been observed in only two other cases.[3] The proband, a girl, was born at term by spontaneous vertex delivery after an uneventful pregnancy. Birth weight was 3500 g, length 55 cm, occipitofrontal circumference (OFC) 35.5 cm, and chest circumference 33 cm, all above the 50th centile. Apgar score was 9 at one minute. Clinical investigation at birth showed preaxial polydactyly of the right hand, which has been surgically corrected. No other malformations were noted at that time. She was the first child of healthy, unrelated parents. At her birth the mother and the father were 24 and 25 years old, respectively. In a following pregnancy, a healthy son was born. Besides a slight delay in beginning to walk independently, at the age of 18 months, the patient reached other early developmental milestones at the normal time. She received all appropriate vaccinations, including live polio, without incident. After the age of 2 years, she experienced several episodes of upper and lower respiratory tract infections, as well as otitis media, but admission to hospital has never been required. The patient was referred to the cardiology department for evaluation of a heart murmur. Patent ductus arteriosus (PDA) was detected and surgically closed at the age of 3 years. At the age of 5 years, she was admitted for evaluation of developmental delay. Metabolic screening including thyroid status was normal. On physical examination at the age of 5.75 years, several dysmorphic features were noted (fig 1A, B) including hypertelorism, epicanthic folds and ptosis of the right eyelid, a short and

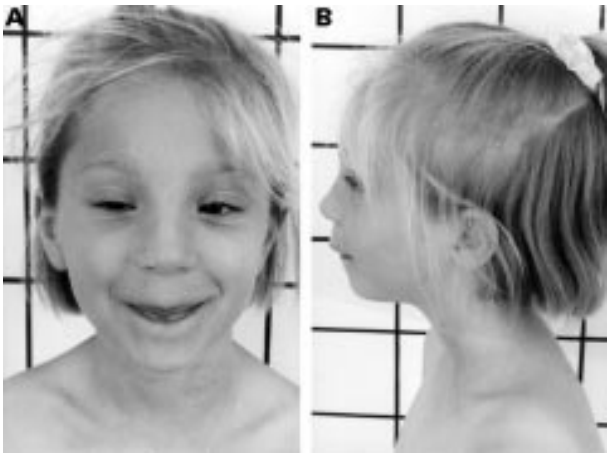

Figure 1 (A, B) The patient aged 6 years.

slightly upturned nose with a high and broad root, an elongated, flat, and prominent philtrum, and a small chin. The ears and hair were normal. Highly placed thumbs, with thickness of the right one, bilateral 5th finger clinodactyly, and partial syndactyly of the 2nd/3rd toes were found. Two naevi flammei and hirsutism in the lumbar area were present. Her measurements at this time were as follows: height 112.5 cm (~25th centile), weight 17.9 kg (~25th centile), and OFC 51 cm (~50th centile), with cranial width less than length (dolichocephaly). A retrospective analysis of her growth parameters showed that her height and weight had usually followed the 50th-25th centile (fig 2A, B); her OFC was constantly around the 50th centile (fig 2C), although the anterior fontanelle was small from birth and closed at the age of 8 months. Her intellectual development assessed at the age of 4 years with the Terman-Merrill test indicated borderline intelligence (IQ=76). Ocular examination showed no abnormalities apart from strabismus. A very detailed neurological evaluation also showed no pathological signs.

A first assessment of humoral immunity, at the age of 6 years, showed a low serum IgA level (0.06 g/l) and slightly decreased level of IgM (0.55 g/l), while a normal concentration of total IgG (5.49 g/l) "masked" deficiency of IgG2 (0.53 g/l) and IgG4 (0.019 g/l). Serum AFP level and blood lymphocyte count were within the normal limits. Lymphocyte subpopulation analysis showed a decreased T cell population (CD3+) because of helper cells (CD4+) deficiency (8%, normal value 40-60%), in particular of virgin helper cells (1.2%). Suppressor T cells (CD8+) were within the normal range (26%) and NK cells were increased (34.3%, normal value 5-10%). T cell response to PHA stimulation was very poor. Three attempts to perform chromosome analysis from PHA stimulated lymphocytes failed. In a one year interval metaphases from two successful cultures were analysed by standard harvesting and GTG banding. From the first culture, 11 of 20 mitoses showed a normal female karyotype 46,XX, eight cells exhibited unique aneuploidies, and one cell carried a translocation (7;14). In the second successful culture, only 14 mitoses could be analysed, including four incomplete metaphases. In two metaphases, an inv(7) and an add(7q) were each detected once. Summarising the data of the two investigations, in three of 34 (~9%) analysed cells rearrangements of chromosome 7 and/or 14 could be

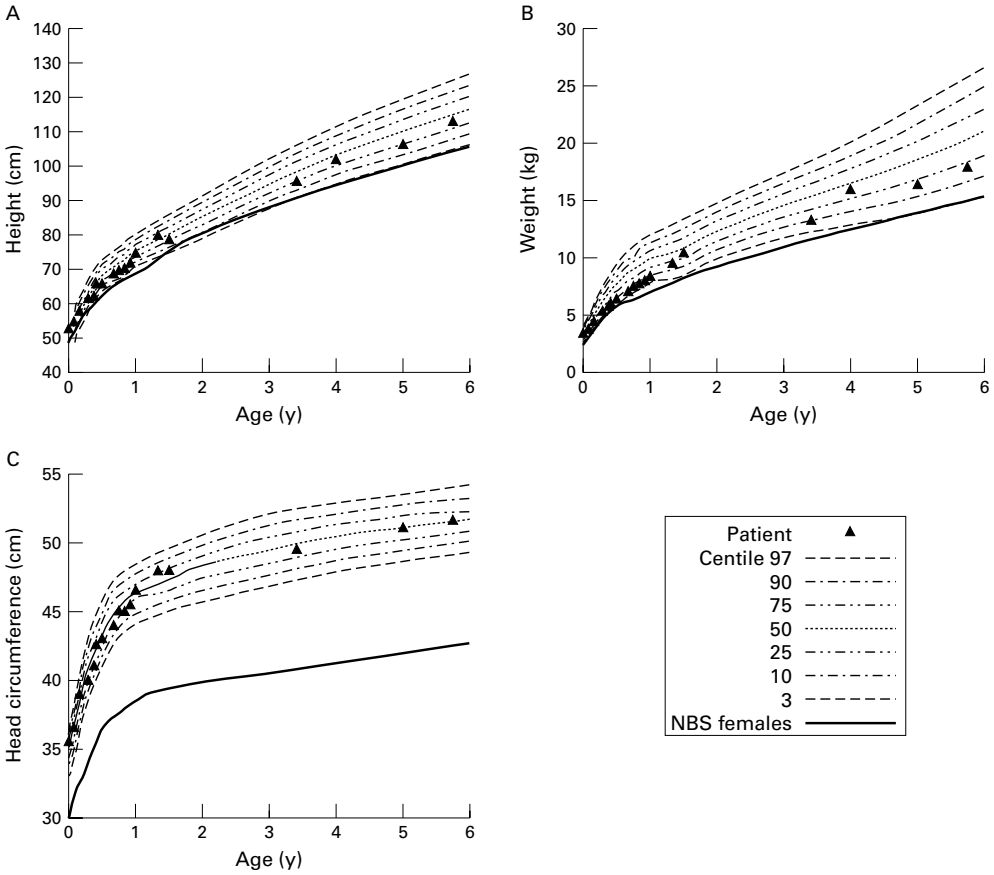

Figure 2 Height (A), weight (B), and head circumference (C) of the patient in relation to normal standards and to the measurements of the 23 NBS females.

detected. Spontaneous chromosomal instability in association with thumb duplications and presence of patent ductus arteriosus (PDA) are also present in Fanconi anaemia (FA) patients. Because FA shows extreme clinical heterogeneity (including a very wide range of onset of anaemia) and considerable overlap with the phenotypes of a variety of other genetic diseases,[6] we decided to exclude a FA mutation in our patient by mitomycin C (MMC) hypersensitivity testing. A lymphoblastoid cell line (LCL) from the index patient, a NBS patient, and a healthy control were treated 24 hours before harvesting with MMC. From each LCL culture, 50 Giemsa stained metaphases were analysed for chromosome aberrations from coded slides. Moderately increased aberration rates were detected in the patients and the NBS cells in response to MMC treatment (table 1). This moderate MMC sensitivity is much lower than in FA patients, but is also found in cell lines from other NBS and AT patients (M Stumm, unpublished data). In a further cytogenetic experiment, LCLs from the index patient, a NBS patient, and a healthy control were *x* irradiated in G2 phase at room temperature. The cells were harvested four hours after irradiation. The cells from the patient showed a marked increase of aberrant metaphases and breaks/cells as compared with control cells. The frequency of aberrant cells and chromosome aberrations was a little higher than in the NBS reference cell line (table 2).

In summary, the patient's cells showed moderate sensitivity to MMC, but marked radiosensitivity, a common cytogenetic hallmark of AT and NBS homozygotes.[2 3] AT and NBS patients also display a similar type of combined immunodeficiency and specific chromosomal rearrangements that frequently involve chromosomes 7 and 14. Our patient had a low IgA concentration, a profound functional defect of T cells with compensatory increase in NK activity, and in 9% of T lymphocytes rearrangements involving chromosome 7 and 14 were found. Therefore, the main cell biological findings common to AT and NBS were confirmed, although the patient did not have some of the cardinal clinical features of NBS (micro-

Table 1 Spontaneous and mitomycin C (MMC) induced chromosomal aberrations in the index patient, a NBS patient, and a control. The cells were cultured for the last 24 hours before harvesting in medium containing the bifunctional alkylating agent MMC

MMC	*ng/ml*	*% aberrant metaphases*	*Breaks/cell*
Control	0	8	0.08
	50	10	0.12
	100	8	0.12
NBS	0	8	0.12
	50	14	0.18
	100	26	0.34
Patient	0	4	0.06
	50	30	0.46
	100	28	0.43

Table 2 Spontaneous and radiation induced chromosomal instability in the index patient, a NBS patient, and a control. The cells were irradiated four hours before harvesting in the G2 phase of the cell cycle

Irradiation	*Gy*	*% aberrant metaphases*	*Breaks/cell*
Control	0	2	0.02
	0.5	8	0.10
	1.0	16	0.18
NBS	0	4	0.06
	0.5	47	0.82
	1.0	68	1.84
Patient	0	6	0.08
	0.5	50	0.98
	1.0	69	2.08

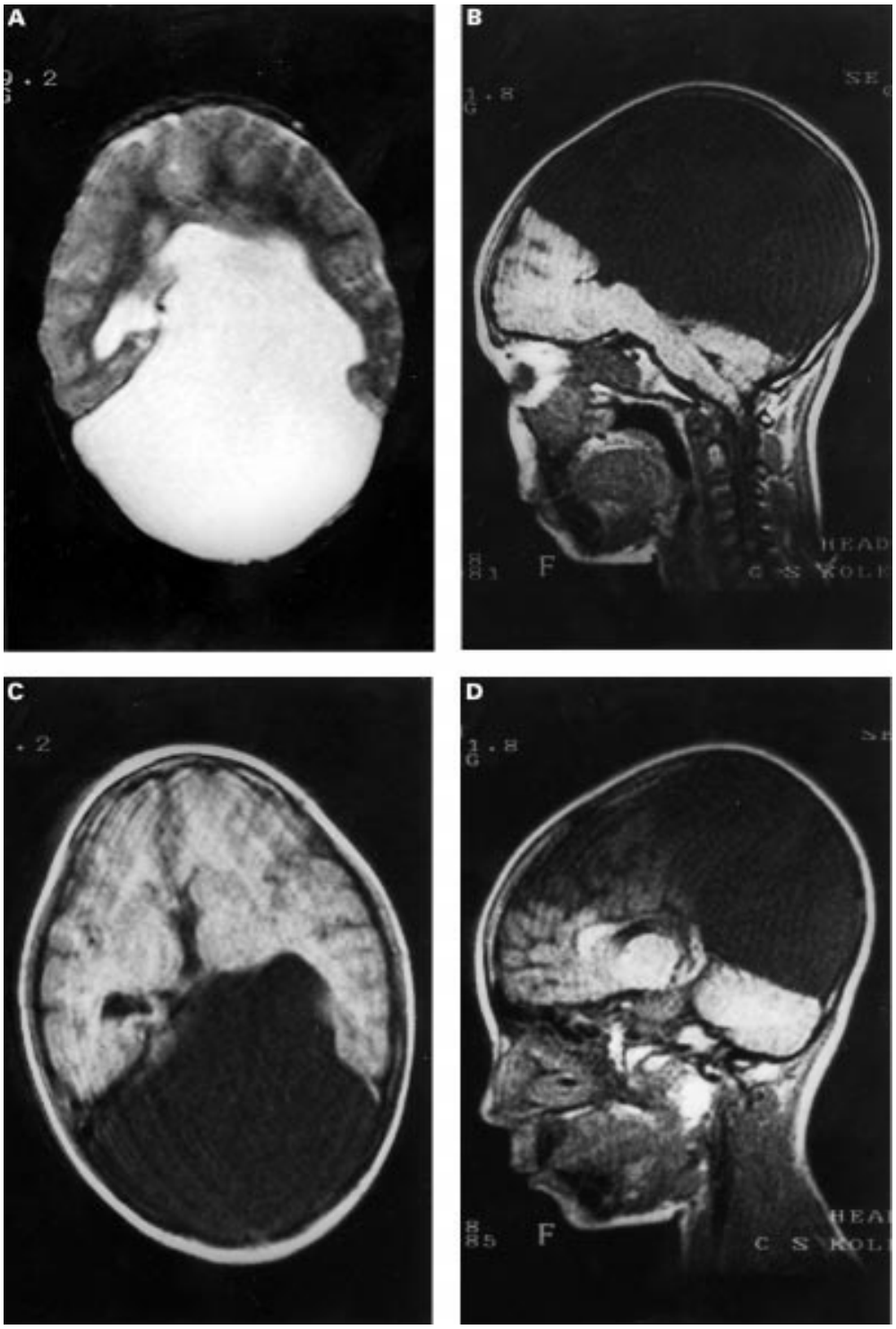

Figure 3 MRI scan of the patient. (A) Large CSF space communicating with the left lateral ventricle and underdevelopment of the parietal lobes. (B) Compression of the posterior fossa; note lack of cerebellar atrophy. (C) Decreased size of the frontal lobes and narrow frontal horns of the lateral ventricles. (D) Partial defect of the corpus callosum.

cephaly and growth retardation, fig 2 shows her measurements compared with NBS females) or the classical AT phenotype. However, as several variants of AT with milder clinical or cellular characteristics of the disease have been reported occasionally,[7] an AT variant with late onset of ataxia was also considered.

The results obtained by cranial MRI were very helpful in further differential diagnostic considerations. A large cyst ($15.0 \times 11.5 \times 10.5$ cm) in the posterosuperior part of the brain, communicating with the left lateral and the third ventricle, was observed (fig 3A). Underdevelopment of the adjacent brain structures (occipital and parietal lobes, posterior part of the left temporal lobe) as a result of their compression by the cyst was found. The cerebellum, in particular its left hemisphere, was also compressed, but otherwise normal, showing no sign of atrophy (fig 3B). Decreased size of the frontal lobes with narrow frontal horns of the lateral ventricles (fig 3C), sinusitis, and

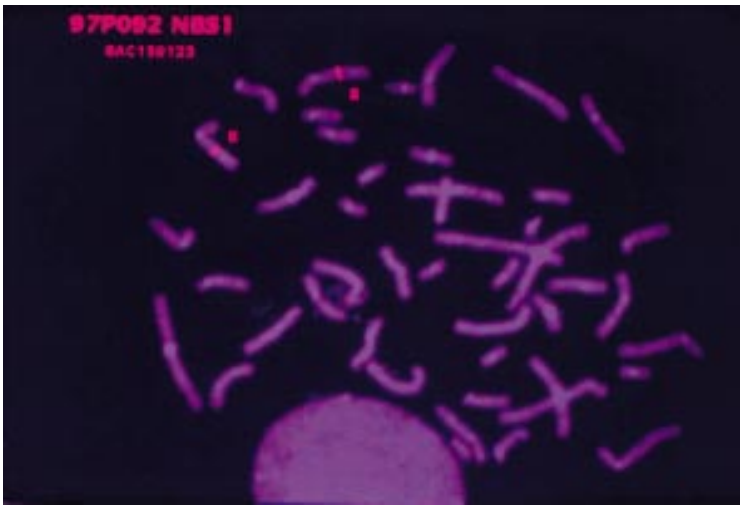

Figure 4 FISH on a DAPI stained LCL metaphase from the patient shows specific hybridisation signals for the Cy3 labelled BAC159I23 on both chromosomes 8, excluding a NBS1 deletion.

absence of the posterior part of the corpus callosum (splenium and part of the truncus, fig 3D), accompanied by a dilated trigon and temporal horn of the right ventricle, were shown. MRI detected a very large, abnormal parieto-occipital cerebrospinal fluid collection, which might explain the lack of microcephaly. Together with the decreased size of the frontal lobes and narrow frontal horns of the lateral ventricles, the diagnosis of NBS was favoured.[8] More particularly, no signs of cerebellar atrophy typical of AT were seen.[9] To date, we have performed cranial MRI in a further 16 NBS patients. All of them had microcephaly. Five also showed partial agenesis of the corpus callosum accompanied by colpocephaly, similar to the girl described, and, in addition, one patient had two large arachnoid cysts. In the latter patient, the decreased OFC was much less prominent (−2.92 SD) than in the remaining group (from −4.0 to −9.62 SD). Hydrocephalus, occipital cyst, and schizencephaly were each reported once in NBS patients after CT scanning.[10–12] Although information on the neuropathology in this disease is very scanty, it appears that the above mentioned developmental abnormalities of the brain may be more common than expected and underdiagnosed in NBS, because patients are frequently asymptomatic. This was the case in the index patient, who never manifested any neurological dysfunction or increased intracranial pressure. However, the anterior fontanelle of the index patient, although always small, closed several months later than usual in patients affected by NBS.[3]

The recent cloning of the *NBS1* gene provided the opportunity to confirm our clinical diagnosis by molecular analysis. The patient was homozygous for the 657del5 mutation in exon 6, the most frequent mutation in NBS patients. Both parents were heterozygous for the same mutation. Fluorescence in situ hybridisation (FISH) with a BAC probe (BAC159I23), containing the whole *NBS1* gene region, was performed on metaphases from the patient. This investigation was necessary to exclude a contiguous gene deletion syndrome in the *NBS1* region, because molecular mutation analysis does not allow discrimination between a homozygous mutation and a hemizygous mutation with an associated deletion of the second allele. A deletion, containing genes surrounding the *NBS1* gene, could result in a variant phenotype. Nevertheless, all metaphases investigated showed normal signal patterns on both chromosomes 8 (fig 4). Therefore, a deletion of the *NBS1* gene could be excluded.

Our report illustrates the clinical variability in Nijmegen breakage syndrome. From our experience, it seems important to point out that neurodevelopmental abnormalities in NBS patients can result in a variable phenotype, as shown in our patient. Therefore, we recommend central nervous system imaging by magnetic resonance imaging as a valuable diagnostic procedure in the evaluation of the NBS patients.

This work was supported in part by grants from the State Committee for Scientific Research of the Republic of Poland: (4P05E07314) to KHC and (4P05B01311) to MBF

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