Electronic letter

Nijmegen breakage syndrome in a Dutch patient not resulting from a defect in *NBS1*

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EDITOR—Nijmegen breakage syndrome (NBS) is a rare autosomal recessive chromosomal instability disorder characterised by microcephaly, immunodeficiency, α ray hypersensitivity, and predisposition to malignancy. The gene responsible for NBS, *NBS1*, is located on chromosome 8q21 and encodes a protein called nibrin. This protein is a component of the hMre11/hRad50 protein complex, suggesting defective DNA double strand break (DSB) repair or cell cycle checkpoint function in NBS.¹⁻⁷ In this report we describe a patient with the NBS phenotype, typical cytogenetic presentation with aberrations in chromosomes 7 and 14, and increased α ray sensitivity. Our index patient had deafness unlike all the other NBS patients reported so far. Mutation detection did not show a mutation in *NBS1* and the protein nibrin was normally expressed.

Case report

The boy is the second child of non-consanguineous parents. His older sister is healthy. From the sixth month of pregnancy microcephaly and growth retardation were noted. Amniocentesis was performed followed by a cytogenetic study which showed a normal male karyotype. He was born in 1997 at 39 weeks of gestation. Birth weight was 1915 g, length 42 cm, and head circumference 28.5

cm (all below the 3rd centile). The neonatal period was complicated by mild hypoglycaemia. During the first year of life he suffered from feeding difficulties and vomiting for which no specific cause was found. Developmental milestones were markedly delayed in language skills because of a severe hearing deficit. Motor skills were reached within the normal range. Head circumference remained below the 3rd centile.

At 20 months of age he was examined in our hospital. On examination, he was a small, microcephalic boy with hearing aids on both sides. Head circumference was 40.2 cm, weight 8.2 kg, and height 77 cm. He had large ears, epicanthic folds, and a receding mandible (fig 1). On his back two hyperpigmented spots were noted. Apart from speech delay, neurological examination was normal. EEG showed no abnormalities. A CT scan of the os petrosus showed severe dysplasia of the cochlea. Cerebral MRI showed no structural lesions. The extent of myelinisation was considered normal for age. Routine blood tests were normal, as were endocrinological studies and amino acid levels.

Immunological studies showed IgG4 deficiency, but the immunoglobulins were otherwise normal: IgG 7.41 g/l, IgG1 6.38 g/l, IgG2

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Figure 1 Facial appearance of the patient.

Figure 2 Western blot showing normal level of nibrin. Lane 1 is the size standard. Lanes 2, 3, and 4 are serial dilutions of a normal control B cell line. The lanes contain 5 × 10E5, 2.5 × 10E5, and 1 × 10E5 cell equivalents. All the remaining lanes on the blot contain 5 × 10E5 cell equivalents. Lane 5 is a NBS patient lacking nibrin. Lane 6 is our index patient with normal nibrin expressed. Lanes 7 and 8 are control patients and lane 9 is a patient with ataxia telangiectasia.

0.89 g/l, IgG3 0.60 g/l, and IgG4 not detectable; IgA 0.68 g/l; IgM 0.80 g/l; IgE 17 U/ml. T cells were within the normal range and both percentages and absolute numbers of CD3, CD4, and CD8 cells were normal. The in vitro response of peripheral blood lymphocytes to the mitogen phytohaemagglutinin was depressed. Specific antibodies after immunisation (diphtheria toxoid, tetanus toxoid, and *Haemophilus influenzae* B) were normal.

Cytogenetic studies showed in 50 cells one typical translocation 7;14 (t(7;14)(p13;q11)) and one inversion 14 (q11;q32). Moreover, 88% of cells showed multiple chromosomal aberrations after exposure to x rays (1.0 Gray) compared to 18% of cells from a normal control.

Mutation detection using genomic DNA was performed by PCR with specific primers for the human *NBS1* gene, followed by automated DNA sequence analysis of the entire open reading frame of both DNA strands. No mutations or polymorphisms were observed in the *NBS1* gene of our index patient.

In western blotting experiments, all *NBS1* mutations in NBS patients are predicted to result in truncation of the protein product nibrin.⁴ For this reason, western blotting was used to assess the quantity and quality of nibrin expressed in lymphoblasts from the patient (fig 2). A normal level of correctly sized nibrin was detected, consistent with the results of the molecular genetic studies.

Discussion

NBS belongs to the family of DNA repair disorders. Other members are Bloom syndrome (BS) and ataxia telangiectasia (AT). All of these disorders show chromosomal instability, immunodeficiency, and predisposition to cancer, but are genetically heterogeneous, involved in different pathways of DNA repair. The gene for *NBS1* has been identified on chromosome 8q21, the gene for BS (*BLM*) on chromosome 15, and the gene for AT (*ATM*) on chromosome 11.³⁻⁶ Apparently, different proteins encoded by different genes play a role in cell cycle control. Although identification of the genes has been very helpful, the specific pathophysiological mechanisms have still to be elucidated.

The patient described here fulfils the criteria for NBS, namely microcephaly, growth retardation, immunodeficiency (although very mild), typical chromosome 7 and 14 aberrations, and x ray hypersensitivity (table 1). His face with receding mandible and epicanthic folds is in keeping with the typical appearance, which usually becomes more obvious with age. At the molecular and protein level, however, the patient shows completely normal results and thus does not fit into the NBS spectrum as described previously. This strongly suggests the presence of another gene, a NBS-like gene. Another family with the NBS phenotype, but without linkage to chromosome 8 has been described by Cerosaletti *et al*⁶ (table 1). The proband had microcephaly, growth retardation, unusual facial features, mild radial cataracts, and diffuse, abnormal skin pigmentation. Deafness was not mentioned. Immunoglobulin levels and B and T cells were normal as in our patient. In NBS nearly all patients have disturbances of serum immunoglobulins and T cell subpopulations.

Hearing loss is seen in many syndromes with microcephaly, but until now it has not been reported in NBS.⁷ Many congenital disorders have been found in some, but not all patients of the group with the Slavic mutation.⁷ The finding of hearing loss may be coincidental. However, specific congenital anomalies such as hearing loss in our patient and cataract in the patient of Cerosaletti *et al*⁶ may be symptoms of other NBS disorders. Identification of the gene involved (*NBS2*) will be an important step forward in unravelling these questions.

Table 1 Clinical and laboratory data

	Our patient	Cerosaletti <i>et al</i> ⁶	NBS reports
Microcephaly	+	+	+
Growth retardation	+	+	+
Mental retardation	+	–	+
Typical facial appearance	+	+	+
Skin abnormalities	+	+	+
Deafness	+	–	–
Cataract	–	+	–
IgG (g/l)	Normal	Normal	Decreased
IgA (g/l)	Normal	Normal	Decreased
IgM (g/l)	Normal	Normal	Decreased
CD3 cells	Normal	Normal	Decreased
CD4 cells	Normal	Normal	Decreased
CD8 cells	Normal	Normal	Normal
NK cells	Normal	Not determined	Increased
Chromosomal instability			
7/7 rearrangements		+	+
7/14 rearrangements	+		+
14/14 rearrangements	+		+
Radiation hypersensitivity	+	+	+

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