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CASE REPORT/OLGU SUNUMU

Nijmegen-Breakage Syndrome; Two Siblings Presenting with Different Phenotypes

Nijmegen Breakage Sendromu; Farklı Fenotipte İki Kardeş

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ABSTRACT

The Nijmegen Breakage syndrome (NBS) is characterized by chromosomal instability, combined immunodeficiency, and distinctive physical features. We present two siblings with NBS presenting with strikingly different manifestations. The proband is a 6-year-old female with short stature, microcephaly, hepatosplenomegaly, rectovaginal fistula, anal atresia, an ectopic kidney, recurrent fevers and otitis media. A 7-year-old brother has developmental delay, failure to thrive, and microcephaly without recurring infections. Both patients have hypogammaglobulinemia, B cell lymphopenia and reduced phytohaemagglutinin-induced lymphocyte proliferation. Both siblings are homozygous for the c.657_661delACAAA (p.Lys219Asnfs*16) mutation in the NBN (NBS1) gene.

Key words: Nijmegen breakage syndrome, lymphopenia, chromosomal instability, microcephaly, pachygyria

ÖZ

Nijmegen Breakage sendromu (NBS); kromozomal instabilite, kombine immün yetmezlik ve ayırd edici fiziksel özelliklerle karakterizedir. Burada farklı klinik belirtiler gösteren iki kardeş sunulmaktadır. İndeks olgu olan 6 yaşında kız hastada boy kısalığı, mikrosefali, hepatosplenomegali, rektovajinal fistül, anal atrezi, ektopik böbrek, tekrarlayan ateş ve otit mevcuttu. Yedi yaşında erkek kardeşinde ise tekrarlayan enfeksiyon öyküsü olmaksızın gelişme geriliği, nöromotor gerilik ve mikrosefali saptandı. Her iki kardeşte de hipogammaglobulinemi, B hücre lenfopenisi ve fitohemaglütinin ile lenfosit proliferasyon yanıtında azalma gözlendi. Her iki hastada NBN (NBS1) geninde homozigot c.657_661delACAAA (p.Lys219Asnfs*16) mutasyonu saptandı.

Anahtar kelimeler: Nijmegen breakage sendromu, lenfopeni, kromozomal instabilite, mikrosefali, pakigiri

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INTRODUCTION

The Nijmegen Breakage syndrome (NBS, MIM 251260) is an autosomal recessive chromosomal instability syndrome with impaired cell cycle checkpoints and DNA double-strand break repair. It is classified under combined immunodeficiencies with associating distinctive features such as microcephaly, characteristic face, short stature, mild intellectual disability, and predisposition to malignancies (1,2). Hypomorphic mutations in *NBN* (MIM 602667), encoding nibrin, are responsible for NBS; null *NBN* mutations are expected to be lethal (3,4). In this report, we present two siblings with the same mutation but with different phenotypes.

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CASE PRESENTATIONS

Two siblings; a 6-year-old girl (PI: patient I) and a 7-year-old boy (PII: patient II) were born to non-consanguineous parents originating from the same village.

PI: The proband was born at 36 weeks gestation weighing 2400 g. Prenatal ultrasound showed microcephaly. She was found to have anal atresia, rectovaginal fistula, and left ectopic pelvicaliectatic kidney at birth. During infancy she had recurrent fevers and otitis media along with urinary tract infections. Bilateral suburethral teflon injection was performed due to severe vesicourethral reflux. Diaper dermatitis and nail dystrophies were associated findings. At age 4 years her weight, height, and head circumference measured 12 kg (3rd centile), 96 cm (between the 3rd and 10th centiles), and 39.5 cm (<3rd centile; -10 SD). Her findings at 6 years of age included short stature, microcephaly, sloping forehead, large ears, long philtrum, hepatosplenomegaly, and prominent superficial veins on physical examination (Figure 1A). Low IgG, IgA and IgE levels with B cell lymphopenia were documented (Table I). Phytohaemagglutinin (PHA) induced lymphocyte proliferation was slightly reduced (29%) compared to an age matched healthy control (36%) (Figure 1B). Denver developmental screening test at 4 years of age showed severe delays in social contact, language, fine and gross motor skills compatible with 15 months, 27 months, 24 months and 30 months of age, respectively. Partial agenesis of corpus callosum, simplified gyral pattern; pachygyria, microcephaly and frontal lobe atrophy, bilateral dilatation of lateral ventricle, partial agenesis of septum pellucidum were documented in cranial magnetic resonance imaging (Figure 2A-E). Etiology of hepatosplenomegaly is being currently investigated, unfortunately not concluded to a specific cause yet. Viral agents, autoimmune markers are all found to be negative. The portal Doppler ultrasonography revealed coarsened liver echotexture suggesting cirrhosis. A diagnostic liver biopsy is currently planned.

Thorax computed tomography showed mediastinal lymph nodes and a 3 mm nodule but no sign of bronchiectasis. At 6 years of age intravenous gammaglobulin and trimethoprim sulfamethoxazole prophylaxis were initiated.

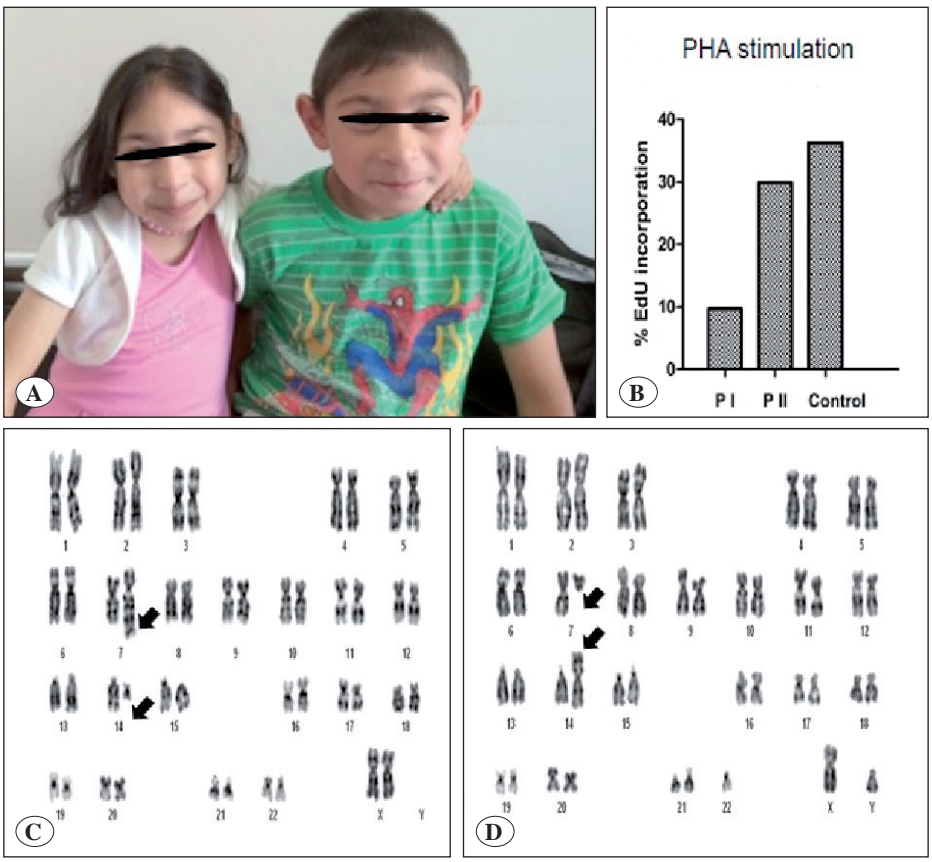

Figure 1. A) Facial dysmorphism and microcephaly,
B) Lymphocyte proliferation assay by phytohemagglutinin induced 5-ethynyl-2'-deoxyuridine (EdU) incorporation,
C) 72 hour phytohemagglutinin induced instability of chromosomes 7 and 14 leading to translocation at t(7;14)(q32;q13), **D)** at t(7;14)(q11;p12) shown by black arrows for both patients.

PII: He was born at 28 weeks gestation weighing 1400 g. Microcephaly was documented at birth without any associated findings. He has been free of infections. At 5 years of age his weight, height, and head circumference were 15 kg (10th centile), 106 cm (25th centile), and 41 cm (<3rd centile; -8 SD), respectively. He had severe developmental delay, prominent superficial veins, microcephaly, large ears and long philtrum. Mild hypogammaglobulinemia and B cell lymphopenia were documented (Table I) although lymphocyte proliferation response to PHA was severely reduced (9%) compared to an age matched healthy control (36%) (Figure 1B). Cranial MRI revealed mild hypoplasia of both frontal lobes.

Cytogenetic analysis of both patients revealed spontaneous chromosome instability in response to PHA but not to diepoxybutane (DEB). 72 hour PHA induced chromosomal analyses for both patients showed new rearrangements; inversions in 7p13 and 7q35, translocations in t(7;14)(q11;p12) and t(7;14)(q32;q13) (Figure 1C,D).

Microcephaly, immune deficiency and growth retardation is a phenotype specific for DNA repair defects. In this line, patients samples were undergone whole exome sequencing (WES) to identify specific disease among a compound set of genetic variations which may cause the same phenotype by using Agilent Sureselect capture kit and Illumina HiSeq 2000 instrument. A homozygous previously reported *NBN* (*NBS1*) NM_002485.4:c.657_661delACAAA (p.Lys219Asnfs*16) variant was identified in both patients and confirmed by Sanger sequencing. Both siblings had homozygous truncating 5 bp deletion 657_661 delACAAA in the NBN gene. Both parents were carrier for the mutation. The family received genetic counseling regarding NBS as well as an increased risk for breast cancer in carriers. The variant NM_002485.4:c.657_661delACAAA submitted to ClinVar (Submission ID: SUB1187971) (http://www.ncbi.nlm.nih.gov/clinvar/).

DISCUSSION

Primary immune deficiencies with mutations resulting in DNA double strand breaks repair share similar features such as neurodegeneration, radiosensitivity and cancer predisposition. DBSs are mainly generated during V(D)J rearrangements hence DNA repair is mandatory for this process in addition to class switch recombination (2).

Characteristic features of NBS include microcephaly, short stature, immunodeficiency, predisposition to malignancies; urogenital anomalies and various hematological disorders may accompany the disease. The dysmorphic facial features include sloping forehead, micrognathia, prominent midface, large dysplastic ears, long nose and upward slanting of palpebral fissures. One of our patients exhibited anal atresia in addition to pelvicaliectasia; which is known to worsen the clinical picture with recurrent urinary system infections (2).

Severe microcephaly is the hallmark of the syndrome in addition to intellectual disabilities and motor problems. Approximately 75% of the patients are microcephalic at birth (5). Despite microcephaly, 50% of patients exhibit normal mental development, moderate retardation may be seen in 10%, though (2). Both of our patients presented with severe developmental delays. Reported central nervous system malformations in NBS include aplasia or hypoplasia of corpus callosum, communicating hydrocephalus and neuronal migration disorders (6,7). PI in our report had pachygyria and agenesis of corpus callosum whereas PII had only mild hypoplasia of frontal lobes. Pachygyria is not a frequent finding in NBS patients but previously reported in a few patients (8,9).

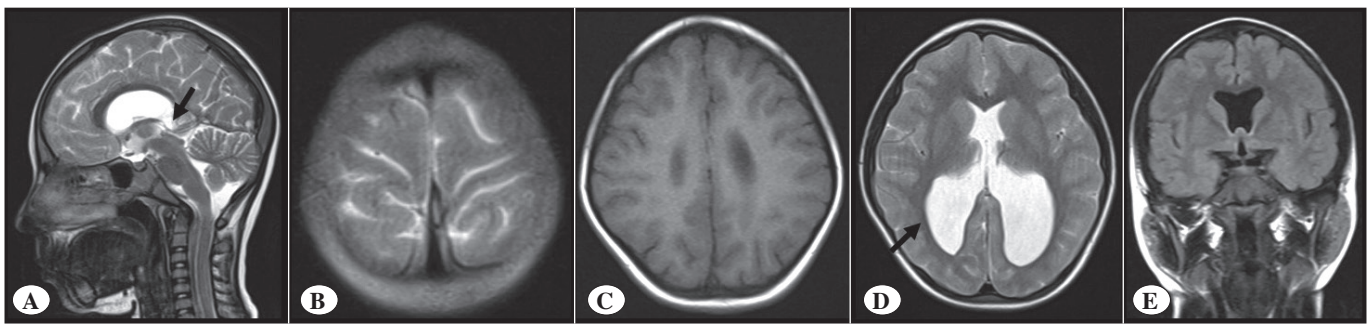

Figure 2. Cranial magnetic resonance imaging of the brain: **A)** partial agenesis of corpus callosum, **B)** simplified gyral pattern; pachygyria, **C)** microcephaly and frontal lobe atrophy, **D)** bilateral dilatation of lateral ventricles, **E)** partial agenesis of septum pellucidum.

Table I. Immunologic assessment of the patients (values beyond the references marked in bold)

	PI 6 yrs	Normal range	PII 7 yrs	Normal range
IgG mg/dl	**137**	764-2134	**538**	764-2134
IgM mg/dl	186	69-387	122	69-387
IgA mg/dl	**<6**	70-303	**45**	70-303
IgE IU/ml	**<1**		**<1**	
IgG1 mg/dl	**<72**	468-1262	**382**	468-1262
IgG2 mg/dl	**<48**	85-430	88	85-430
IgG3 mg/dl	**<10**	32-191	68	32-191
ALC/µl	10700	1100-5900	1900	1100-5900
T cells/µl	10165	700-4200	1178	700-4200
$CD4^+$ T cells/µl	1070	300-2000	665	300-2000
Naïve $CD4^+$ T cells (%)	**11**	53-86	**28.3**	46-77
Memory $CD4^+$ T cells (%)	**99**	9-26	**77.6**	13-30
$CD4^+CD31^+CD45RA^+$ T cells (%)	**7.1**	41-81	**4.2**	41-81
$CD8^+$ Tcells/µl	8560	300-1800	551	300-1800
Naïve $CD8^+$ T cells (%)	**6**	69-97	**46.9**	63-92
Memory $CD8^+$ T cells (%)	**96**	4-16	**46.6**	4-21
NK cells /µl	267	90-900	494	90-900
B cells/µl	**132**	200-1600	**105**	200-1600
Naïve B cells (%)	77	47.3-77	37.4	47.3-77
Class-switched memory B cells (%)	**4.7**	10.9-30.4	17	10.9-30.4
Unclass-switched memory B cells (%)	16	5.2-20.4	39	5.2-20.4
Transitional B cells (%)	4.9	4.6-8.3	5.3	4.6-8.3
Plasmoblasts (%)	3.64	0.6-5.3	3.3	0.6-5.3
$CD21^{low}$ 38^{low} B cells (%)	**17**	2.3-10	**16.2**	2.3-10
$TCR\alpha\beta^+CD3^+CD4^-CD8^-$ T cells (%)	1.07		1.68	
$TCR\alpha\beta^+CD3^+$ T cells (%)	88	44-92	89.6	44-92
$TCR\gamma\delta^+CD3^+$ T cells (%)	9.8	2-24	8.66	2-24
$CD3^+HLADR^+$ T cells (%)	56	3-14	17.6	3-14

Although lymphocyte counts are known to be low in NBS, PI showed lymphocytosis. Both patients exhibited hypogammaglobulinemia except for IgM levels, which is generally expected to be higher in NBS due to defective class switching. Our patients showed low CD4, B and naive CD4 cells as seen in previously reported patients (10). The naive/memory disproportion was also detected in CD8 T cells. Our patients showed impaired lymphocyte proliferation when stimulated with PHA as reported in NBS patients (10).

Chromosome instability can be detected in stimulated T lymphocytes of patients with NBS, specific inversions and translocations are frequently observed between the loci of the immunoglobulin and T-cell receptor genes on chromosomes 7 and 14 (11,12). The most frequent aberrations are observed in chromosome bands 7p13, 7q35, 14q11, 14q32 (13) that was present in both of our patients.

Microcephaly, immune deficiency and growth retardation is a phenotype specific for DNA repair defects including NBS, NBS like disease, Bloom syndrome, Cernunnos deficiency, Ataxia-telangiectasia (AT), AT like disease, NHEJ1, ICF and DNA ligase 4 deficiency.

The c.657_661delACAAA mutation has been reported in 90% of patients with NBS. While the mutation leads

to a frameshift, a protein lacking the native N terminus is produced, which is physically associated with the MRE11 complex for DNA repair. It has been shown to be a founder mutation in Slavic populations (14,15). The same mutation has been reported in multiple families with NBS from Turkey (16,17). Five Turkish families having affected children with NBS who were homozygous for the 5 bp *NBN* deletion were reported (PMID: 12123493 and PMID: 16392640). All reported Turks with the mutation shared the same haplotype that was reported from the Slavic individuals carrying the same mutation. The c.657_661delACAAA mutation was not present in 402 Turkish controls, suggesting that it is not common in Turkey (PMID: 16392640).

Girard et al. (18) reported variations of cells bearing the same 657del5 mutation, suggesting that differences in the 'genetic background' may affect the cellular phenotype. Although 657del5 mutation is seen in 90% of the patients, the clinical course varies among the same mutated NBS patients. But it is still interesting to find out two sibs presenting different phenotypes especially when similar genetic background is taken into account, modifier genetic factor may cause this discrepancy.

Our experience with the two siblings from the same family shows wide clinical heterogeneity of NBS. Delayed diagnosis may lead to associated malignancies, therefore multidisciplinary approach is mandatory to decrease morbidity and mortality in those families.

CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

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