Supplemental Data

Human RAD50 Deficiency in a Nijmegen Breakage Syndrome-like Disorder

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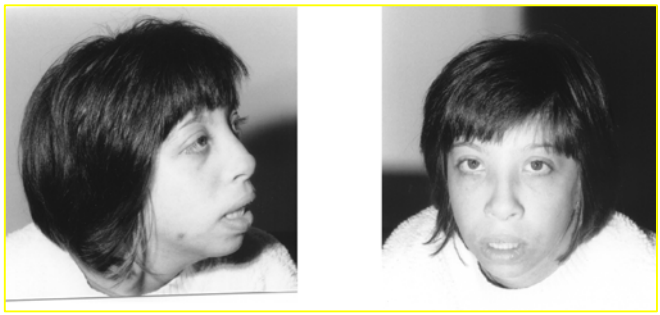
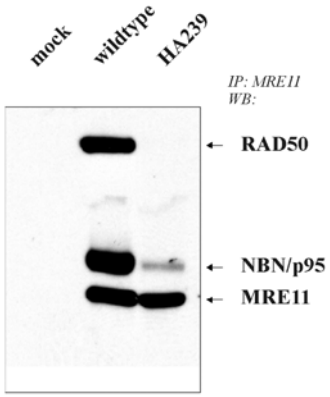
Figure S1. Presentation of the Patient with RAD50 Deficiency



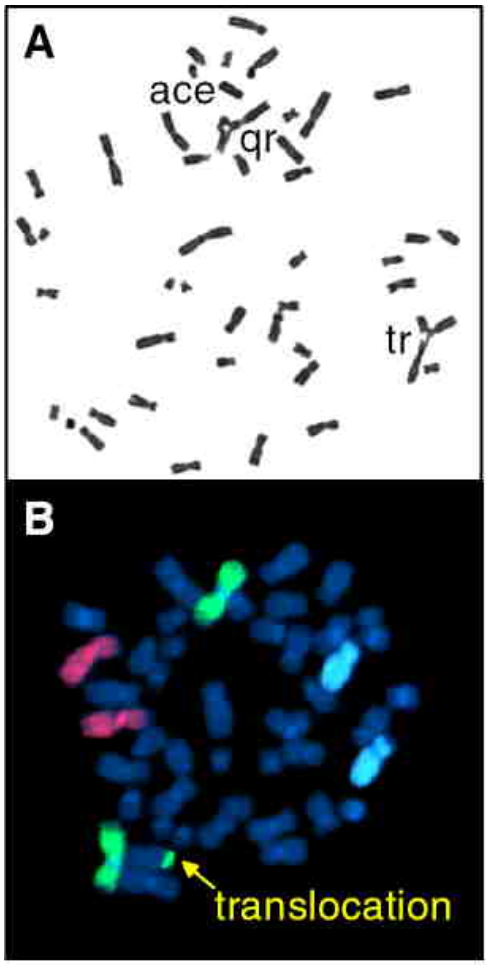
Photograph of the patient at age 15 showing NBS-typical facies and microcephaly. For comparison, note that photographs of the patient during her first 4 years of age have been published previously[1].

Figure S2. MRE11-NBN Interaction in RAD50-Deficient Cells



Extracts were prepared from wild-type and RAD50-deficient LCLs (HA239) and immunoprecipitated with anti-MRE11 antibody prior to immunoblotting with antibodies against RAD50, MRE11 and NBN as indicated.

Figure S3. Chromosomal Aberrations of RAD50-Deficient Cells without Irradiation



(A) Cytogenetic analysis of Giemsa stained metaphases from Rad50-deficient lymphocytes showed spontaneous chromatid exchanges (chte). **qr** = asymmetrical quadriradial interchange with complete exchanges and acentric fragment (**ace**); **tr** = symmetrical triradial interchange with incomplete exchanges. (B) An example of whole chromosome painting of chromosomes 1 (green), 2 (red) and 4 (light blue) revealing a spontaneous translocation involving one of the chromosomes 1.

Table S1. Primer Sequences and Genomic PCR Conditions to Amplify 25 Exons of the *RAD50* Gene

5'-AACAGCACCCAGCACCTAGC-3' and 5'-CCTACACCTGTGGAGCCCTA-3'	(exon 1, annealing 59°C)
5'-GGTAAACTTCTGTGGTTCTC-3' and 5'-GGTTACTACTGGGTGCTAAA-3'	(exon 2, annealing 53°C)
5'-CCAACACTGGTGCTTATTAA-3' and 5'-CAGTACTTTCTGCCCAATTT-3'	(exon 3, annealing 51°C)
5'-AACGGGATAGGTGAAGGGCC-3' and 5'- GGTTGCTGCTGGATGAGAGG-3'	(exon 4, annealing 59°C)
5'-GTGACAGCATAATATCCCAC-3' and 5'-TTTAGCCAGTCCACGATGTA-3'	(exon 5, annealing 53°C)
5′-CAGCCATGTAAGCTATAGTGAG-3′ and 5′-ATGTGGATGGCAAAATGGATTC-3′	(exon 6, annealing 61°C)
5′-GATCATATTTTCTTATGTTTGTAC-3′ and 5′-CATCATAGTAGGAAAAACGACC-3′	(exon 7, annealing 56°C)
5′-CTCGTGAATCTGCAGCTATCTC-3′ and 5′-CAATTCAAGTAGAGAATATGATGC-3′	(exon 8, annealing 56°C)
5′-TTCTTCAGTGTACATATATTCC-3′ and 5′-GAACCAAAGAGTCAGAAGATCAAG-3′	(exon 9, annealing 56°C)
5′-CCTTAGAGCATATATAGTGCC-3′ and 5′-GGCTATTTTAAGTACCAGACAG-3′	(exon 10, annealing 61°C)
5′-GATATAATGTGGAGATATAGAC-3′ and 5′-CAGGTAAGCATGAAATAAGAG-3′	(exon 11, annealing 56°C)
5′-GGTCATACCAAACTCTTGTC-3′ and 5′-CAAAGGTGTCAAAGTATCCTG-3′	(exon 12, annealing 61°C)
5′-ACAACCGTATTCAGAATACTG-3′ and 5′-CTGCTACATGTACAGTGAAGG-3′	(exon 13, annealing 61°C)
5′-GAACACAATGTCACTTCTGTGG-3′ and 5′-CCTGTACCTGAATACTAGCTAC-3′	(exon 14, annealing 61°C)
5′-AAGATTTTGAATAATGCAGTAAG-3′ and 5′-CATGTGCTCGCAATGTCAAAGTC-3′	(exon 15, annealing 56°C)
5′-GCATTTGTGGATTCCATAGACC-3′ and 5′-CCTGGGTGACAGAACGAGACTG-3′	(exon 16, annealing 56°C)
5′-GAGCCTGGCACATAGAAAGTG-3′ and 5′-GACGTGGTGCTATGAACATAAG-3′	(exon 17, annealing 61°C)
5′-CCTGTTATGTGCCCTTAAGTAC-3′ and 5′-GCATTTCTATTCAATGGATCTTC-3′	(exons 18-19, annealing 61°C)
5′-GTCACCAGTTGCCTGTTACAG-3′ and 5′-CTTCACATTCCAGTAATAAAGAC-3′	(exon 20, annealing 56°C)
5′-TCTATGACTTTTCCACTTCAGG-3′ and 5′-ACTGCAATAAGAAAATCCCCAG-3′	(exon 21, annealing 58°C)
5′- CCAAGCAGCAAAGTTTTGCTGCTG-3′ and 5′- CATGATGAGAGGTCATAAGGGG -3′	(exon 22, annealing 56°C)
5′-GCTACAGAGCATAGGTTCCTC-3′ and 5′-CTCTTTCAGTTACTTGGGTGAG-3′	(exon 23, annealing 56°C)
5′-CCCTGCTGAAAAGATCATGTC-3′ and 5′-GTGAGATACTTACTCAACCAG-3′	(exon 24, annealing 56°C)
5′-GCACAAGTTCATGTGTCTGAC-3′ and 5′-ATACACTTTCTGAGGACCTAC-3′	(exon 25, annealing 60°C).

Table S2. Clinical and Laboratory Findings of the RAD50-Deficient Patient

CLINICAL FINDINGS
Congenital abnormalities ● NBS-like facies ● Microcephaly (<3rd percentile) ● Pre- and postnatal growth retardation ● Very subtle and non-progressive ataxia ● Mild spasticity ● Skin hyper- and hypopigmentation, ∅ 15 mm ● Hyperopia
Immunological status ● No severe or recurrent infections ● Normal Ig status (IgG,A,M,D and IgG subclasses) ● Normal lymphocyte counts ● Normal alpha-fetoprotein levels

Table S3. Spontaneous and Radiation-Induced Chromosomal Breakage Rates in RAD50-Deficient, NBS, and A-T Cells

	0 Gy		**0.5 Gy**		**1.0 Gy**	
	%[a]	b/m[b]	%[a]	b/m[b]	%[a]	b/m[b]
Patient blood	24	0.54	76	2.78	90	3.98
Patient LCL	10	0.14	62	1.12	91	2.15
NBS[c] blood	12	n=1 0.16	60	n=1 1.16	74	n=1 2.16
NBS[c] LCL	9± 7	n=24 0.14±0.15	55±11	n=11 1.16±0.32	70±12	n=11 2,38±0.88
AT LCL	11±4	n=8 0.18 ±0.10	69±14	n=6 1.52±0.29	82±17	n=6 2,78±1.06
Controls blood	1±2	n=96 0.02 ±0.02	15±8	n=50 0.19±0.11	22±8	n=50 0,27±0.10
Control LCL	2±3	n=38 0.03±0.04	14±8	n=18 0.19±0.15	23±9	n=18 0,35±0.18

Giemsa-stained metaphases from lymphocyte cell cultures and lymphoblastoid cell lines (LCL) were analysed as indicated. n= number of patients or controls analysed in each cohort. 50 metaphases were analysed for each patient/ control. [a]Percentages of aberrant metaphases; [b]breaks/metaphase; [c]all tested NBS cells had truncating mutations in *NBN* [2].

Supplemental References

1. Barbi, G., Scheres, J.M., Schindler, D., Taalman, R.D., Rodens, K., Mehnert, K., Müller, M., and Seyschab, H. (1991). Chromosome instability and X-ray hypersensitivity in a microcephalic and growth-retarded child. Am. J. Med. Genet. *40*, 44–50.
2. van der Burgt, I., Chrzanowska, K.H., Smeets, D., and Weemaes, C. (1996). Nijmegen breakage syndrome. J. Med. Genet. *33*, 153–156.