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Fine Localization of the Nijmegen Breakage Syndrome Gene to 8q21: Evidence for a Common Founder Haplotype

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Summary

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disorder characterized by microcephaly, a birdlike face, growth retardation, immunodeficiency, lack of secondary sex characteristics in females, and increased incidence of lymphoid cancers. NBS cells display a phenotype similar to that of cells from ataxia-telangiectasia patients, including chromosomal instability, radiation sensitivity, and aberrant cell-cycle–checkpoint control following exposure to ionizing radiation. A recent study reported genetic linkage of NBS to human chromosome 8q21, with strong linkage disequilibrium detected at marker D8S1811 in eastern European NBS families. We collected a geographically diverse group of NBS families and tested them for linkage, using an expanded panel of markers at 8q21. In this article, we report linkage of NBS to 8q21 in 6/7 of these families, with a maximum LOD score of 3.58. Significant linkage disequilibrium was detected for 8/13 markers tested in the 8q21 region, including D8S1811. In order to further localize the gene for NBS, we generated a radiation-hybrid map of markers at 8q21 and constructed haplotypes based on this map. Examination of disease haplotypes segregating in 11 NBS pedigrees revealed recombination events that place the NBS gene between D8S1757 and D8S270. A common founder haplotype was present on 15/18 disease chromosomes from 9/11 NBS families. Inferred (ancestral) recombination events involving this common haplotype suggest that NBS can be localized further, to an interval flanked by markers D8S273 and D8S88.

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Introduction

Nijmegen breakage syndrome (NBS; MIM 251260) is a rare autosomal disorder. The NBS patient registry, maintained in Nijmegen, currently contains entries for 53 patients, many of whom are of eastern European descent. The clinical features of the disease include microcephaly from birth, a birdlike face, growth retardation, immunodeficiency in both humoral and cellular immune responses, susceptibility to respiratory-tract infections, lack of secondary sex characteristics in females, and an increased incidence of lymphoid cancers, primarily lymphomas (van der Burgt et al. 1996; Chrzanowska et al. 1997). In vitro, NBS cells are sensitive to radiation and radiomimetic compounds (Taalman et al. 1983). Peripheral blood lymphocytes display spontaneous and radiation-induced chromosomal instability that frequently involves the immunoglobulin and T-cell receptor loci on chromosomes 7 and 14 (Weemaes et al. 1981; Taalman et al. 1989). Following exposure to ionizing radiation, NBS cells are unable to activate the G1 cell-cycle checkpoint and are unable to halt progression through the S phase, a feature termed "radioresistant DNA synthesis" (Jaspers and Bootsma 1982; Murnane and Painter 1982; Taalman et al. 1983; Jongmans et al. 1997; Sullivan et al. 1997). This latter phenotype has been used to classify NBS cells into two complementation groups, V1 and V2 (Jaspers et al. 1988; Wegner et al. 1988).

Many of the features of NBS are similar to the more common chromosomal-instability disorder ataxia-telangiectasia (AT). Like NBS, AT patients are characterized by radiation sensitivity, radioresistant DNA synthesis, chromosomal instability preferentially involving chromosomes 7 and 14, immunodeficiency, and increased incidence of lymphoid cancers (Gatti 1998). As a result, NBS initially was thought to be a clinical variant of AT, connected by the very rare AT-Fresno syndrome, which has features of both NBS and AT (Curry et al. 1989). However, substantial evidence now indicates that

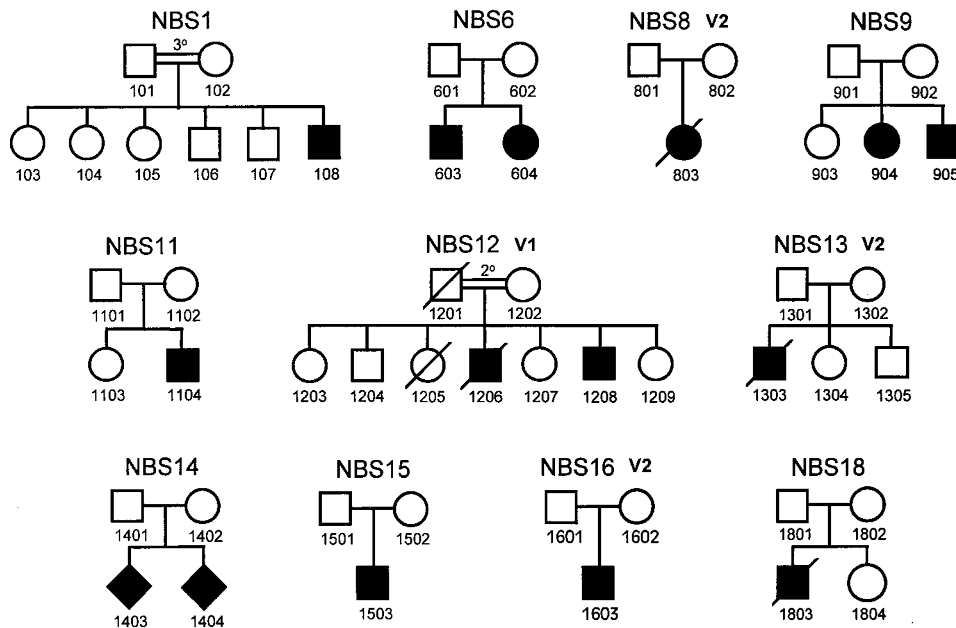

Figure 1 NBS pedigrees. Eleven families with characteristic symptoms of NBS were ascertained from the United States, Poland, Britain, Spain, The Netherlands, Germany, and Canada. A family's complementation-group assignment, V1 or V2, is indicated if known. For families NBS1, NBS6, and NBS16, DNA was available only from the affected individuals. For family NBS18, DNA was available from individuals NBS1801 and NBS1803.

NBS is genetically distinct from AT. This evidence includes the observations that fusion of AT and NBS fibroblasts results in complementation of radioresistant DNA synthesis; that transfer of a normal chromosome 11, encoding the ATM gene, into NBS cells does not complement their radiation sensitivity; and that haplotype analysis excludes linkage of NBS to the AT region at 11q23 (Jaspers et al. 1988; Wegner et al. 1988; Stumm et al. 1995; Komatsu et al. 1996). In addition, we found no mutations in the ATM gene in NBS affecteds, using SSCP analysis (authors' unpublished data).

Recently, Matsuura et al. (1997) mapped the gene mutated in NBS to human chromosome 8, using microcell-mediated chromosome transfer. These investigators found that chromosome 8 complemented radiation sensitivity in both NBS V1 and V2 fibroblasts, which is consistent with the notion that cells from the two different complementation groups have defects in the same gene. Analysis of deletion hybrids localized the gene to 8q21-24. Coincidentally, Saar et al. (1997) reported linkage of NBS to marker D8S1811 on chromosome 8q21 in 14 eastern European families, with a maximum LOD score of 6.86 at a recombination fraction of zero. A 1-cM minimal region for the NBS gene was identified between markers D8S271 and D8S270, on the basis of genetic recombinants. Strong allelic association was reported at D8S1811, suggesting a founder effect for a common mutation in this population.

We collected a geographically diverse group of NBS families—from the United States, Canada, England, The Netherlands, Germany, Poland, and Spain—and tested these families for linkage to chromosome 8. In this article, we report linkage of NBS to 8q21 in 6/7 NBS families; furthermore, there is strong evidence of linkage disequilibrium at D8S1811 and surrounding markers. Generation of a radiation-hybrid map at 8q21 allowed us to perform haplotype analysis of 10 markers. Our results identified both a conserved haplotype among NBS patients and ancestral recombinants that further localize the NBS gene to a region between D8S273 and D8S88.

Subjects and Methods

NBS Families

A total of 11 NBS families were collected for this study (fig. 1). Families NBS6, NBS8, NBS9, NBS12, NBS13, and NBS18 were collected from Poland (NBS6), the United States (NBS8), Britain (NBS9), The Netherlands (NBS12 and NBS13), and Canada (NBS18) and have been described elsewhere (Weemaes et al. 1981; Conley et al. 1986; Taalman et al. 1989; Chrzanowska et al. 1995; Green et al. 1995; Der Kaloustian et al. 1996). Family NBS1 was ascertained from the United States, by P.K. Families NBS11 and NBS16 were collected by

R.A.G. Family NBS11 is from Spain, and family NBS16 was ascertained from the United States. Families NBS14 and NBS15 were ascertained from Germany, by B.S. and B.H.B., and are of Slavic ancestry. All the patients had symptoms consistent with a diagnosis of NBS, including microcephaly, growth retardation, immunodeficiency, chromosomal instability, and radiation sensitivity and/or radioresistant DNA synthesis.

Blood samples were obtained from the families by informed consent, and DNA was prepared by standard procedures (Sambrook et al. 1989). For families NBS1, NBS6, and NBS16, DNA was available only from the affected individuals. For family NBS18, DNA was available from the father and the affected. In addition, DNA was limiting for individual NBS1104. To allow analysis, DNA from NBS1104 was amplified randomly by a primer-extension–amplification protocol (Zhang et al. 1992) using 25 ng genomic DNA, 40 μM random 15mers, 100 μM dNTPs, 10 mM Tris pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, and 5 U *Taq* DNA polymerase (Boehringer Mannheim). PCR was accomplished by means of 50 cycles of 95°C for 1 min, 37°C for 2 min, and 55°C for 4 min, with a ramp from 37°C to 55°C of 10 s/°C. For genotyping reactions, 2.5 μl of the resultant PCR product was used.

Families NBS1, NBS6, NBS9, NBS11, NBS12, NBS13, and NBS14 were used for linkage studies. The remaining families—NBS8, NBS15, NBS16, and NBS18, which were not informative for linkage—were included in the linkage-disequilibrium and haplotype analyses.

Genotyping

NBS families were genotyped for 13 microsatellite markers from a 15.3-cM region at 8q21 (table 1). Markers were assayed by PCR using 20 ng genomic DNA; 0.33 mM spermidine; 50 mM Tris pH 9; 20 mM $(NH_4)_2SO_4$; 1.5 mM $MgCl_2$; 40 μM each of dTTP, dGTP, and dCTP; 4 μM dATP; 0.1 μCi α[^{33}P]-dATP (Amersham); 0.6 μM each primer; and 0.5 μl *Taq* DNA polymerase. Amplification was accomplished by use of a touchdown protocol of 65°C–55°C, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. PCR products were analyzed on 5% acrylamide/8 M urea denaturing gels by use of 1 × Tris borate–EDTA buffer (89 mM Tris borate/2.5 mM EDTA). Electrophoresis was performed at 50 mA. Gels were dried following electrophoresis and were autoradiographed. Alleles were sized relative to CEPH control 134702, for each marker.

Linkage Analysis

Multipoint linkage analysis was performed by use of the GENEHUNTER linkage-analysis program (Kruglyak et al. 1996), under the assumptions of a fully penetrant autosomal recessive disease, a disease-allele frequency of .001, and no phenocopies. Allele frequencies were estimated from the NBS parents. The genetic map order used was D8S286–2 cM–D8S1757–5.5 cM–(D8S271, D8S1707, D8S273)–1.1 cM–(D8S88, D8S1724, D8S1811, D8S1146, AFMa041xg5, D8S270, D8S1818)–6.7 cM–D8S506 and was based on the Généthon map (Dib et al. 1996).

Table 1

Microsatellite Markers Genotyped at 8q21

Marker	Genetic Distance[a] (cM)	Heterozygosity[b]
D8S286	2	.80
D8S1757	5.5	.78
D8S271	0	.76
D8S1707	0	.69
D8S273	1.1	.79
D8S1724	0	.48
D8S1811	0	.62
D8S88	0	.84
AFMa041xg5	0	ND
D8S1146	0	ND
D8S270	0	.79
D8S1818	6.7	.61
D8S506	…	.69

[a] Estimated distance from the next marker.
[b] ND = not determined.

Linkage-Disequilibrium Analysis

Linkage disequilibrium was assessed by use of the transmission-disequilibrium test (Spielman et al. 1993). Transmissions of alleles from heterozygous NBS parents to affected offspring were tabulated for each of the markers genotyped in families NBS8, NBS9, NBS11, NBS12, NBS13, NBS14, NBS15, and NBS18. In the case of family NBS18, in which only the father and the proband were available for testing, transmissions were scored only if the proband was neither heterozygous for the same alleles as the parent nor homozygous at a given marker. This resulted in a maximum of 19 informative transmissions that could be scored at each marker. Significant evidence of transmission disequilibrium was assessed by performance of a two-sided Fisher's exact test.

Radiation-Hybrid Mapping

A radiation-hybrid map spanning D8S271–D8S1818 was generated by scoring for the presence of markers D8S271, D8S1707, D8S273, D8S88, D8S1724, D8S1811, D8S1146, AFMa041xg5, D8S270, and D8S1818 in the Stanford G3 and TNG3 radiation-hybrid panels (Research Genetics). The G3 radiation-hybrid panel was generated with 10,000 rads of ionizing radiation, and the TNG3 panel was generated with 50,000 rads of radiation. For each marker, 35 or 25 ng of DNA from each of the hybrids (G3 or TNG3 panels,

respectively) and the human and rodent controls was amplified with 0.6 μM each primer, 0.33 mM spermidine, 50 mM Tris pH 9, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 40 μM each dNTP, and 0.5 μl *Taq* DNA polymerase. Amplification was accomplished by use of a touchdown protocol of 65°C–55°C, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. PCR products were analyzed by agarose-gel electrophoresis and were scored as "positive," "negative," or "undetermined." Analysis of the retention patterns of the markers in the TNG3 and G3 hybrid panels was accomplished by performance of a joint analysis using the RHMAP program (Lunetta et al. 1996), under the assumptions of a common retention probability for all fragments in the same panel and nonproportional intermarker distances in different panels.

Haplotype Analysis

Haplotypes were constructed for the affecteds in all the NBS families, on the basis of the following map order: D8S286-D8S1757-D8S1707-D8S273-D8S1724-D8S1811-D8S88-D8S270-D8S1818-D8S506. The locations of D8S286, D8S1757, and D8S506 were set on the basis of the genetic map, whereas D8S1707, D8S273, D8S1724, D8S1811, D8S88, D8S270, and D8S1818 were ordered by radiation-hybrid mapping. To identify an ancestral haplotype, disease chromosomes bearing the 15 allele at D8S1811 were compared, taking into account the allelic association observed at markers throughout the region. For families NBS6 and NBS16, where the parents were unavailable for determination of the phase of the chromosomes in the affecteds, the phase was constructed to maintain the ancestral haplotype over the largest region of the chromosome.

Results

Linkage to Markers at 8q21 in the NBS Families

A total of 11 families—from the United States, Canada, England, Spain, The Netherlands, Germany, and Poland—with characteristic symptoms of NBS were ascertained (fig. 1). In some cases, the complementation-group status of a family had been determined; however, families were grouped together for analysis irrespective of their complementation-group assignment, on the basis of the observation that chromosome 8 complements both V1 and V2 NBS fibroblasts (Matsuura et al. 1997).

Saar et al. (1997) previously reported linkage to markers on chromosome 8q21 in a collection of predominantly Polish NBS families. To confirm and extend these results, 13 microsatellite markers from a 15.3-cM region at 8q21 (table 1) were genotyped in seven of the NBS families in this geographically diverse group. Evidence for linkage was assessed by use of the program GENEHUNTER (Kruglyak et al. 1996). Figure 2 shows the results of the multipoint analysis. The maximum multipoint LOD score detected was 3.58. Six of the seven NBS families had positive LOD scores in this region. Linkage to markers at 8q21 was not detected for one consanguineous family, NBS1, represented by a single affected individual. When this family was excluded, a maximum LOD score of 4.28 was obtained. The maximum LOD score was not affected appreciably by changing the map order and map distances to incorporate aspects of the subsequently determined radiation-hybrid map of the region, suggesting that the linkage to 8q21 was robust (range 3.51–3.62).

Linkage Disequilibrium at 8q21

Strong linkage disequilibrium has been observed at marker D8S1811 in eastern European NBS families (Saar et al. 1997). To determine if allelic association could be detected in our collection of NBS families, a transmission-disequilibrium test was performed (table 2). Eight of the 13 markers at 8q21 that were genotyped in these families showed significant evidence of linkage disequilibrium ($P < .01$). Striking linkage disequilibrium was detected at markers D8S1724, D8S1811, and D8S88 ($P < .0001$). The peak linkage disequilibrium appeared to be at D8S1811, where the 15 allele was trans-

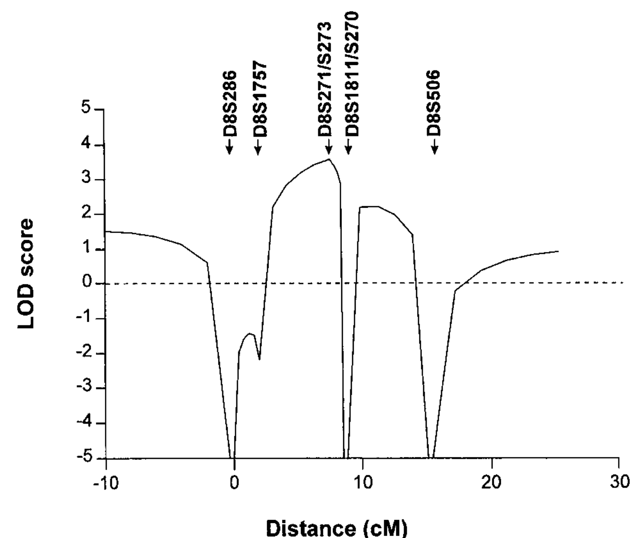

Figure 2 Multipoint linkage analysis of the NBS locus and markers at 8q21. Seven NBS families—namely, NBS1, NBS6, NBS9, NBS11, NBS12, NBS13, and NBS14—were genotyped for 13 microsatellite markers at 8q21. Multipoint linkage analysis was performed by use of the GENEHUNTER linkage-analysis program. Map order and distances were obtained from the Généthon genetic map. "D8S271/S273" represents the cluster of markers D8S271-D8S1707-D8S273, and "D8S1811/S270" represents the cluster of markers D8S88-D8S1724-D8S1811-D8S1146-AFMa041xg5-D8S270-D8S1818.

Table 2

Transmission-Disequilibrium Test for Markers at 8q21 in NBS Families

Marker and Status	No. of Each Allele									Total No. of Alleles	P Value[a]
D8S286:	1	3	5	6	7	8	9	10	12		
Transmitted	1	4	0	7	4	0	0	2	0	18	.0145
Not transmitted	0	2	1	2	4	5	3	0	1	18	
D8S1757:	1	6	7	10	11	12	15				
Transmitted	2	3	7	0	3	2	0			17	.0001
Not transmitted	0	5	0	7	0	3	2			17	
D8S271:	1	2	4	5	6	7	8				
Transmitted	4	3	0	0	2	4	2			15	.0052
Not transmitted	4	0	2	1	8	0	0			15	
D8S1707:	1	4	5								
Transmitted	2	5	4							11	.4374
Not transmitted	4	2	5							11	
D8S273:	1	2	3	4	5	7					
Transmitted	3	1	7	0	0	1				12	.0788
Not transmitted	5	2	1	2	1	1				12	
D8S1724:	4	5	6	7	9						
Transmitted	2	0	0	11	1					14	<.0001
Not transmitted	1	1	12	0	0					14	
D8S1811:	2	3	5	12	14	15					
Transmitted	4	0	0	0	1	14				19	<.0001
Not transmitted	12	1	1	4	1	0				19	
D8S88:	4	5	6	7	8	9	11	12			
Transmitted	2	2	0	0	13	0	1	0		18	<.0001
Not transmitted	3	2	6	2	0	2	0	3		18	
AFMa041xg5:	1	2	5								
Transmitted	6	0	0							6	.0022
Not transmitted	0	5	1							6	
D8S1146:	1	2	3	4	5						
Transmitted	2	8	0	0	0					10	.0073
Not transmitted	1	2	1	1	5					10	
D8S270:	1	2	5	6	7	8					
Transmitted	0	1	2	1	6	9				19	.0001
Not transmitted	4	1	5	6	3	0				19	
D8S1818:	1	2	3								
Transmitted	4	3	4							11	1.0000
Not transmitted	4	3	4							11	

(*continued*)

Table 2 (continued)

Marker and Status	No. of Each Allele				Total No. of Alleles	*P* Value[a]
D8S506:	1	2	3	4		
Transmitted	1	7	6	0	14	.0192
Not transmitted	4	1	6	3	14	

[a] Calculated by use of a two-sided Fisher's exact test.

mitted exclusively to affected offspring a total of 14 times. This allele corresponds to the 108-bp allele previously reported to be in linkage disequilibrium in eastern European NBS families (Saar et al. 1997). In addition to these three markers, strong linkage disequilibrium also was detected at D8S1757 and D8S270 ($P = .0001$).

Generation of a Radiation-Hybrid Map at 8q21

The genetic map at 8q21 fails to order many markers in the region, particularly between D8S1757 and D8S1818, where the greatest evidence for linkage to NBS was detected (fig. 2). In order to improve the resolution of the genetic map of this region, genotype data from our NBS families were combined with genotype data from 11 CEPH families. However, this approach did not refine the map significantly.

As an alternative approach, we generated a radiation-hybrid map at 8q21. Markers D8S271, D8S1707, D8S273, D8S1724, D8S1811, D8S88, D8S1146, AFMa041xg5, D8S270, and D8S1818 were typed in the Stanford G3 and TNG3 radiation-hybrid panels. These hybrid panels were generated with 10,000 and 50,000 rads of ionizing radiation, to yield resolutions of ~500 kb and ~100 kb, respectively. A joint analysis of the results was performed by use of the RHMAP program (Lunetta et al. 1996). For six of the loci tested, a 1,000:1 framework map was determined as follows: D8S1707-D8S273-D8S1724-D8S88-D8S270-D8S1818 (fig. 3). This framework map was fixed, and each of the four remaining markers was added individually. This analysis placed D8S1811 between D8S1724 and D8S88, at 394:1 odds, but did not provide an unambiguous location for D8S271, D8S1146, and AFMa041xg5. The distances, in centirays (cR), for the map in each of the radiation-hybrid panels are shown in figure 3. The total map length in the G3 panel was 219.8 cR. This corresponds to 8,133 kb, when the average value of 37 kb/cR reported for chromosome 8 in the G3 panel (Stewart et al. 1997) is used, which contrasts with the genetic distance of 1.1 cM for this region.

Identification of a Minimal NBS Region, by Haplotype Analysis

The radiation-hybrid–map order given above was used to define haplotypes in the NBS families, for the purpose of identifying the minimal region that could contain the NBS gene. For this analysis, only those markers that could be ordered with robust odds in the radiation-hybrid map or those that could be ordered in the genetic map were considered. The haplotypes for 14 NBS affecteds are presented in figure 4.

Four recombinant chromosomes were identified in our NBS families. NBS1208 and NBS1303 both had recombination events between D8S1757 and D8S1707, setting the proximal boundary for the NBS gene at D8S1757. For the distal side, NBS603 had a recombination event between D8S88 and D8S270, and NBS905 had a recombination event between D8S1818 and D8S506. These results place the NBS gene in the interval between D8S1757 and D8S270.

Since strong linkage disequilibrium was detected at markers in the NBS region, we specifically compared disease chromosomes bearing the 15 allele at D8S1811. A single conserved haplotype encompassing markers D8S1724 (7 allele), D8S1811 (15 allele), D8S88 (8 allele), and D8S270 (8 allele) was identified (fig. 4), which is consistent with the extent of linkage disequilibrium in the region. For some NBS-patient chromosomes, it was possible to infer ancestral recombination events in the conserved haplotype by identification of markers for which the disease-associated allele has been lost. In order to be conservative in this approach, we did not consider cases such as D8S88 in NBS6 or D8S1811 in NBS1603, for which the disease-associated allele for a single marker has been replaced with an allele differing by only one repeat unit, since these instances simply could represent mutations at the marker locus. In this analysis, NBS803 had an apparent ancestral recombination event between D8S1811 and D8S88, replacing the 8 allele at D8S88 with the 11 allele. Since no linkage disequilibrium was detected at D8S273, these results suggest that the region containing the NBS gene can be limited further to the interval between D8S273 and D8S88, a distance of 64.7 cR in the G3 radiation-hybrid panel.

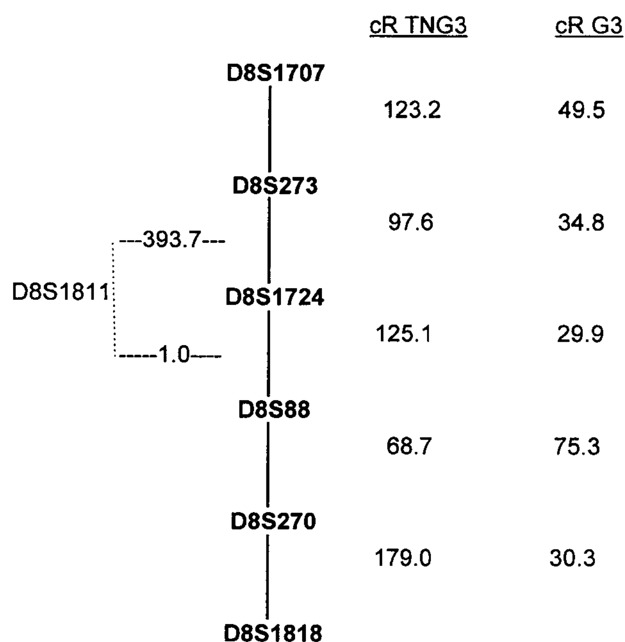

Figure 3 Radiation-hybrid map of markers at 8q21. Ten markers between D8S271 and D8S1818 were typed in the Stanford G3 and TNG3 radiation-hybrid panels, and the results were analyzed by performance of a joint analysis with the RHMAP program. A 1,000:1 framework map was established for the six loci indicated in boldface type. The distances calculated for this map, in both radiation-hybrid panels, are shown to the right. The location of D8S1811, relative to D8S1724, in the framework map is indicated to the left, with the most likely position, between D8S1724 and D8S88, represented by a likelihood ratio of 1.0 and the next-most-favored location represented by a likelihood ratio of 393.7.

In addition to chromosomes bearing the ancestral haplotype, eight chromosomes that did not carry the 15 allele at D8S1811 were identified (fig. 4). Consistent with the absence of linkage of NBS1 to markers at 8q21, NBS108 had unique alleles at D8S1811 (alleles 1 and 11) and was heterozygous for most markers across the region. NBS1503 had one chromosome carrying the 14 allele at D8S1811. This chromosome possibly may bear the ancestral haplotype, since the 8 allele is present at D8S88, and the difference of one repeat unit at D8S1811 could be due to mutation. NBS1303 was homozygous at markers between D8S1707 and D8S506, with the 2 allele at D8S1811. The nonconserved chromosomes in NBS904, NBS905, and NBS1803 also carried the 2 allele at D8S1811. However, there was no evidence of a shared haplotype on these chromosomes. Thus, these chromosomes may carry distinct mutations in the NBS gene.

Discussion

We detected linkage of NBS to markers at 8q21, with a maximum LOD score of 3.58, in seven NBS families from different geographical regions. These results confirm those of Saar et al. (1997), who reported linkage of NBS to markers at 8q21 in 14 eastern European NBS families. Interestingly, one family, represented by a single consanguineous proband (NBS108), did not show linkage to 8q21. This proband was first evaluated at 16 mo of age and was observed to have microcephaly, growth retardation, unusual facial features, mild radial cataracts, and diffuse abnormal skin pigmentation, including café-au-lait spots. He has suffered from recurrent minor infections and fevers, although an initial immunological evaluation revealed normal immunoglobulin levels and no T-cell or B-cell abnormalities. An in-depth cytogenetic analysis showed increased spontaneous chromosomal breakage in primary fibroblast cultures, as well as an increase in chromosomal aberrations following treatment of lymphocytes, with bleomycin. A chromosomal translocation involving chromosome 7, t(7;7)(p15;q36), was observed in phytohemagglutinin-stimulated lymphocytes, although the frequency of the aberrations was within normal limits. Lymphocytes from this individual were radiation sensitive at levels comparable to those for AT homozygotes (Huo et al. 1994). With the exception of the cataracts, these features are typical of NBS. A diagnosis of AT was excluded because of the absence of ataxia, normal serum alphafetoprotein levels, and heterozygosity at microsatellite markers from the ATM locus at 11q23. In addition, we failed to find any mutations in the ATM gene in NBS108, by SSCP analysis. Thus, the absence of linkage to 8q21 in family NBS1 suggests the possibility of genetic heterogeneity in NBS.

Despite the diverse origins of our collection of NBS families, strong linkage disequilibrium was detected at multiple markers in the 8q21 region. In particular, we observed association with the same allele at D8S1811 as that reported for the eastern European NBS families. By mapping and genotyping additional markers in the region, we were able to define a conserved haplotype on chromosomes bearing the 15 allele at D8S1811. Among our 11 NBS families, 6 were homozygous for this haplotype, whereas 3 more were heterozygous. Only one proband (NBS1303) whose family was consistent with linkage to 8q21 did not carry the conserved haplotype on either parental chromosome. These results indicate the presence of a strong founder effect, presumably of Slavic origin, in NBS. It is likely that chromosomes carrying the ancestral haplotype will have a single conserved mutation. In contrast, there was no evidence of a shared haplotype among chromosomes in the remaining NBS families, which did not carry the ancestral haplotype. These chromosomes appear to represent at least four novel NBS mutations.

In their initial report of linkage, Saar et al. (1997) identified an NBS minimal region between D8S271 and D8S270, a 1-cM interval based on the Généthon map.

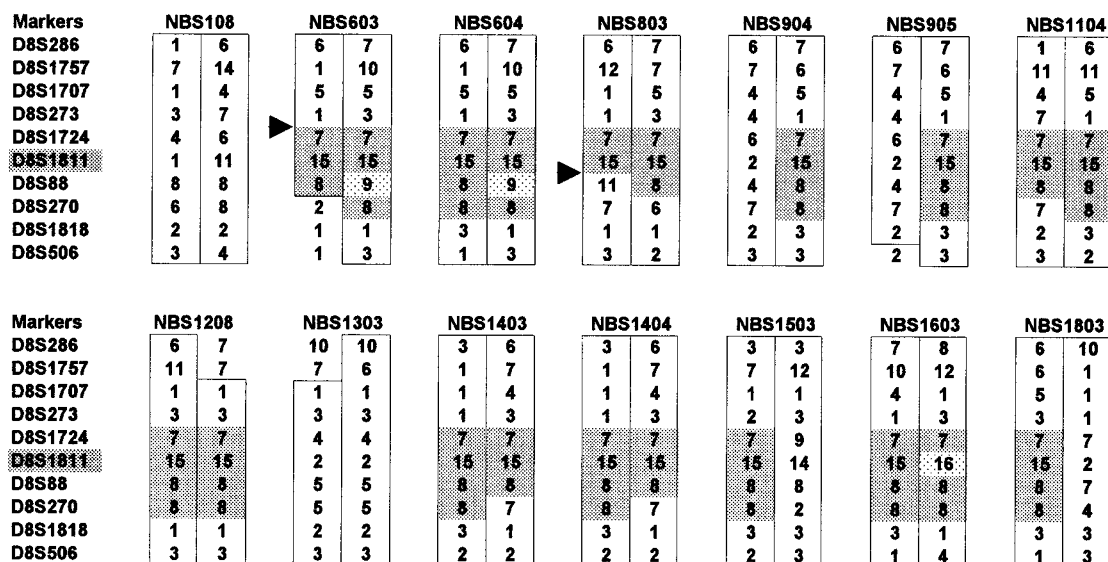

Figure 4 Haplotypes of NBS affecteds, at 8q21. Haplotypes for 14 NBS affecteds were constructed by use of the 10 markers (shown at left) that were ordered unambiguously in the genetic and radiation-hybrid maps. The specific alleles detected at each marker are shown for each individual, and parental chromosomes are boxed. Truncations of the boxes indicate genetic recombination events that excluded linkage. The conserved founder haplotype identified on chromosomes carrying the 15 allele at D8S1811 is indicated by gray shading. Individual markers within this ancestral haplotype that differ by a single repeat length are indicated by stippling. The arrowheads indicate inferred ancestral recombination events that appear to demarcate the minimal region encoding the NBS gene.

We expanded our analysis by genotyping 10 additional markers within the D8S271–D8S270 interval in our NBS families. Haplotypes constructed with these additional markers identified genetic recombinants that place the NBS gene between D8S1757 and D8S270. We were able to limit the region further by identifying a conserved haplotype present on disease chromosomes and inferring ancestral recombination events that altered the distal boundary of the conserved haplotype. These results suggest a new minimal NBS region between D8S273 and D8S88, encompassing the markers D8S1724 and D8S1811. This is a distance of <1 cM, or 65 cR, based on the G3 radiation-hybrid panel.

Our study also provides a more refined map of the 8q21 region. We were successful in ordering 7/10 unordered markers between D8S271 and D8S270 in the genetic map, using radiation-hybrid mapping. This radiation-hybrid map is in agreement with the Whitehead Institute doubly linked contig map WC-449, for those markers tested in common, with one exception: the Whitehead Institute map places D8S88 and D8S1811 proximal to D8S1724. At this time, we cannot distinguish between these two map orders, on the basis of our preliminary physical mapping of the region (Cooper et al. 1997). If the Whitehead Institute order is correct, the NBS minimal region would be between D8S88 and D8S270, but this region still would encompass markers D8S1811 and D8S1724. The most surprising outcome of the radiation-hybrid mapping, however, was the estimated size of the region D8S1707–D8S1818. Although this region is only 1.1 cM in length on the genetic map, the radiation-hybrid analysis generated a length of 220 cR in the G3 radiation-hybrid panel. By use of the average value 37 kb/cR, reported for chromosome 8 (Stewart et al. 1997), this corresponds to a distance of 8.1 Mb. Our physical mapping at 8q21 supports the notion that the region is larger than suggested by the genetic map but not to the extent indicated by the radiation-hybrid mapping (Cooper et al. 1997).

One puzzle that remains to be solved for NBS is why the disease is so rare, with most cases being accounted for by individuals carrying the conserved haplotype on both parental chromosomes. Perhaps an ascertainment bias exists, since the disease is not well known and many individuals with NBS may not have been diagnosed. Currently, the majority of cases in the NBS registry were identified at a small number of clinical centers. It is possible that more cases will be identified as work continues on the mapping and cloning of the gene mutated in NBS. An alternative explanation is that the symptoms associated with NBS are unique to the mutation present on the conserved haplotype. Other mutations in the same gene may cause different symptoms or may be lethal. Identification of new cases with nonconserved haplo-

types, as well as cloning of the gene and generation of knockout animals, may shed some light on this puzzle.

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Electronic-Database Information

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for NBS [MIM 251260])

Whitehead Institute/MIT Center for Genome Research. An STS-based map of the human genome, http://www-genome.wi.mit.edu/cgi-bin/contig/phys_map

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