
In a recent article, Varon et al. (1) described four novel amino acid substitution mutations in the NBS1 gene, NBS1, in 7 of 47 (14.9%) children with relapsed ALL (1). This finding raises important questions about the role of constitutional and somatic mutations in the NBS1 gene in the etiology of childhood leukemia, a disease for which there is only limited evidence of genetic susceptibility. We report that a screen of constitutional (remission) DNA samples from 231 United Kingdom children with primary leukemia, 90 children with primary lymphoma, and 332 normal cord blood samples for the four mutations described by Varon et al. (1) failed to show a significant contribution to susceptibility to these diseases.

NBS (Online Mendelian Inheritance in Man 251260) is a rare, autosomal recessive, AT-like disorder, characterized by genome instability, growth retardation, immunodeficiency, and cancer (usually lymphoma) predisposition (2–4). Although NBS possesses many of the features of AT, including cellular hypersensitivity to ionizing radiation, linkage analysis showed that the gene, located on chromosome 9q21, was on a different chromosome to ATM (5, 6). This was confirmed when NBS1 was cloned (7–9) and found to consist of 16 exons encoding a 754 amino acid, p95 protein known as nibrin (7).

Cytological analysis has revealed that nibrin associates with the products of the MRE11 and RAD50 genes at the site of radiation-induced double-strand DNA breaks (10), the multiprotein MRE11/RAD50/NBRIN complex serving as a sensor-transducer in the ATM/NBS1/SMC1 S-phase checkpoint pathway (11). Malignant diseases, particularly lymphoma and leukemia, have been observed in over a third of NBS patients under the age of 21 years (2), the great majority being associated with the nibrin-truncating mutation 657del5. Indeed, it has been suggested that the increased risk of cancer in the Slavic population could be related to the high frequency of heterozygous 657del5 mutation carriers in this population (12).

To examine the role of the NBS1 mutations described by Varon et al. (1) in childhood leukemia and lymphoma, we screened remission DNA samples from 321 children with primary leukemia and lymphoma and 332 normal cord blood samples. The leukemia patient series comprised 17 children with pro-B ALL, 91 with precursor B (common, pre-B) ALL, 64 with T-ALL, and 59 with AML. The childhood lymphoma series consisted of 39 patients with HL, 21 with B-cell NHL, and 30 with T-NHL. Our study received ethical approval from the Central Manchester (United Kingdom) Local Research Ethics Committee. We used allelic wild-type and variant 17-21-mer SSOPs hybridized to PCR-amplified genomic DNA to detect the NBS1 variants. NBS1 exons 3, 5, and 6 were amplified using primer sequences (Oswal, Southampton, United Kingdom) derived by Varon et al. (8) in PCR mixtures containing Taq polymerase (Life Technologies, Inc., exons 3 and 5) or hot start PlatinumTaq ( exon 6). PCR mixtures consisted of primers, deoxynucleotide triphosphates, Taq polymerase and genomic DNA template, and thermocycling conditions were optimized for each exon. Amplification was verified by electrophoresis of the PCR products in 1.5% agarose gels.

We screened the patient and cord blood DNA samples for S93L and D95N, both encoded by exon 3 of NBS1, 1171V encoded by exon 5, and R215W encoded by exon 6 (1). In addition, we screened the DNA samples for the pathogenic NBS1 mutation 657del5. Appropriate PCR products were hybridized with variant and wild-type SSOP so that each subject could be checked for heterozygosity or homozygosity. Two of the mutations in exon 3 (nt 278C>T and nt 283 G>A, coding S93L and D95N, respectively) were separated by only 4 nucleotides. A panel of four SSOPs spanning both variants was designed to detect either of the two wild-type sequences, one or other of a single wild-type and a single variant sequence or both variant sequences, allowing the identification of all possible genotypes. Although DNA from patients carrying NBS1 mutations was not available to us, we designed complementary single-stranded SSOP as positive control target sequences, tailed them with dTTP, and anchored them to the nylon membranes carrying patient and control PCR products.

The PCR products were arrayed as microspots onto replicate nylon membranes (Biotron Nylon Membrane; ICN Pharmaceuticals, Basingstoke, United Kingdom) using a Biomek 2000 robotic workstation (Beckman Instruments, High Wycombe, United Kingdom), fixed and denatured in situ, prehybridized with Denhardt’s hybridization buffer, and hybridized with [γ32P]-ATP end-labeled SSOP overnight at 50°C–58°C. The membranes were washed, sealed between plastic film, and imaged on an InstantImager (Canberra Packard). Radioactivity detected in each microspot was scored by comparing cpm in test versus control microspots on the same filter and cross-referenced to a real-time bitmap image. In cases (<5) where a DNA sample failed to hybridize with either probe, a check of the filter showed that this was most likely because of insufficient sample.

Our results showed no evidence of S93L or D95N in remission DNA from any of the primary leukemia or lymphoma patients, in contrast to Varon et al. (1) who detected these in the germ-line and in leukemia cells, respectively. However, we did detect D95N in one normal cord blood sample. Unlike Varon et al. (1) who detected I171V in three of seven NBS1 mutation-positive ALL patients, we did not detect this variant in any of our leukemia patients or cord blood samples, but we did detect it in a single HL patient. R215W was reported by Varon et al. (1) to be present in 1 T-ALL patient and in 9 NBS probands of Slavic origin. We detected this variant in 4 leukemia patients (2 c-ALL, 1 T-ALL, and 1 AML), 1 patient with HL, and 2 cord blood samples. The 2.6-fold excess of R215W in the leukemia patients as compared with the cord blood samples was not significant, although it is of interest that this variant is three amino acids upstream from the NBS1 truncating mutation 657del5, close to the breast cancer COOH-terminal domain (7). We found no evidence of the major NBS1 mutation 657del5 in our leukemia-lymphoma patient series, unlike Varon et al. (1) who detected it in 2 of 60 ALL patients, but we did identify it in 2 normal cord blood samples. We found no evidence of any of the variations in the 51 childhood NHLs tested in agreement with others (13–15) that NBS1 mutations make little contribution to sporadic NHL.

All of the case and cord blood samples in which an NBS1 variant was detected in this study were heterozygotes with the wild-type NBS1 allele in agreement with Varon et al. (1). Our results do not support the findings by Varon et al. (1) that the NBS1 mutations mortified for each exon. Amplification was verified by electrophoresis of the PCR products in 1.5% agarose gels.

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Received 1/31/03; revised 4/14/03; accepted 4/30/03.

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1 We thank the United Kingdom Childhood Cancer Study for permission to use patient material and the United Kingdom Department of Health for funding this study.

The abbreviations used are: NBS, Nijmegen breakage syndrome; ALL, acute lymphoblastic leukemia; SSOP, sequence-specific oligonucleotide probe; AML, acute myeloid leukemia; NHL, non-Hodgkin’s lymphoma; HL, Hodgkin’s lymphoma; AT, ataxia-telangiectasia.
identified in their study occur at a relatively high frequency in childhood ALL. This leads us to conclude that these variants play only a minor role, if at all, in constitutional susceptibility to childhood leukemia and lymphoma. It is possible that the reason for the discrepancy between the two sets of results could be because of ethnic differences in the frequency of specific NBS1 variants, although this will require further study. Furthermore, because our analysis was confined to the four polymorphic variants and one pathogenic NBS1 mutation documented by Varon et al. (1), we cannot yet exclude the possibility that there are other variants that could contribute to sporadic childhood leukemia or lymphoma susceptibility in our patient population.

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