CARRIER DETECTION OF WERNER’S SYNDROME USING A MICROSATELLITE THAT EXHIBITS LINKAGE DISEQUILIBRIUM WITH THE WERNER’S SYNDROME LOCUS

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Summary Werner’s syndrome (WS) is a rare autosomal recessive disorder, one of the progeroid syndromes, characterized by features of premature aging. The genetic defect in WS is unknown but recently the genetic linkage of WS to several markers on the short arm of chromosome 8 has been reported. Genetic analysis of 25 families with WS demonstrated that D8S339 was the closest marker linked to the gene locus for Werner’s syndrome (WRN), with a peak lod score of 18.29 at recombination frequency 0.001, and showed a linkage disequilibrium with the WRN locus. We studied two unrelated families with WS using ANK1, D8S339, and D8S360. The mutative haplotype identified through the generations in pedigrees provides a means of carrier detection and presymptomatic diagnosis.

Key Words Werner’s syndrome, molecular diagnosis, microsatellite, carrier detection

INTRODUCTION

Werner’s syndrome (WS) is an autosomal recessive disorder characterized by features of premature aging such as ocular cataracts, graying hair, hypogonadism, cutaneous atrophy, short stature, and osteoporosis. About 10% of patients develop neoplasia, with a particularly high frequency of sarcoma and meningioma. The mean life span of WS patients is 45–50 years and most patients die of malignant neoplasms or various forms of arteriosclerosis. The diagnosis of WS is usually made in the third decade. Skin fibroblasts from WS patients demonstrate slow growth and reduced life span in vitro (Epstein et al., 1966). Recently the WRN
locus was mapped to 8p11.2-p12, and several microsatellite markers such as ANK1, D8S87, and D8S339 were reported to be closely linked to the WRN locus (Goto et al., 1992; Schellenberg et al., 1992; Thomas et al., 1993; Yu et al., 1994). We examined 25 unrelated families with WS using D8S339, and demonstrated that D8S339 was the closest marker linked to the WRN locus and exhibited linkage disequilibrium with the WRN locus. Since heterozygous carriers with WS mutation are apparently normal and healthy, it is difficult to differentiate them from among members of WS families by clinical examination only. In two unrelated families we examined 4 patients and 2 apparently normal siblings. Haplotyping of the WRN region provides a means of carrier detection and presymptomatic diagnosis.

MATERIALS AND METHODS

Subjects and diagnosis. We studied 36 members from 25 Japanese families. In 20 Japanese families, only one first or second cousin patient was analyzed. Two unrelated families were from more complicated inbred pedigrees. In the remaining 3 families, affected and unaffected siblings or both parents were genotyped. Parents of O family (No. 18 family) were from a first cousin marriage. It is not clear whether the parents of H family (No. 22 family) were from a consanguineous marriage. All patients defined as WS satisfied the criteria for WS proposed by Nakura et al. (1994).

Preparation of DNA and PCR assays. DNA was prepared from peripheral blood leukocytes according to standard protocols. We used ANK1, D8S339, and D8S360 for DNA markers on the short arm of chromosome 8 (Polymeropoulos et al., 1991; Thomas et al., 1993; Kamino et al., 1993). These markers are located on the small region with the order cen-ANK1-6cM-(D8S339, WRN)-11.5 cM-D8S360-tel (Nakura, unpublished data). The accurate order of D8S339 and WRN was obscure. We synthesized PCR primers for each marker. PCR reaction was carried out in a total volume of 10 μl containing 50 ng of genomic DNA, 4 pmol of one unlabeled primer, 4 pmol of a 32P-ATP end-labeled primer (0.2 μCi), 200 μM dNTP, 1% formamide, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl at pH 8.4 and 0.25 U Taq polymerase, using Perkin Elmer Cetus Thermal Cycler for 35 cycles as follows: 94°C for 45 sec, annealing temperature for each primer for 30 sec, and 72°C for 30 sec for each cycle. The amplified product was fractionated in a 6% polyacrylamide gel containing 30% formamide and visualized by autoradiography. The size of the alleles was determined by comparison to M13mp18 DNA sequencing ladders.

Linkage analysis and linkage disequilibrium tests. Linkage analysis was performed using the computer program LINKAGE version 5.1 (Lathrop et al., 1984). It was assumed that the gene frequency of WRN was 0.004 and the disease was inherited in an autosomal recessive fashion with complete penetrance (Epstein