Carrier Detection in Wiskott-Aldrich Syndrome: Combined Use of M27β for X-Inactivation Studies and As a Linked Probe

By Judith Goodship, Joan Carter, Teresa Espanol, Yvonne Boyd, Sue Malcolm, and Roland J. Levinsky

Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by immunodeficiency, thrombocytopenia, and eczema. Infections and the hemorrhagic tendency that result from the thrombocytopenia are the main causes of early mortality. There is an increased incidence of lymphoreticular malignancies and leukemias that contributes to later morbidity and mortality. Bone marrow transplantation (BMT) is curative but median survival is less than 10 years without BMT. Female carriers of WAS have no clinical or immunologic abnormalities.

The underlying defect in WAS is not known. The decreased size and number of platelets is a secondary phenomenon as both are corrected by splenectomy. Parkman et al reported the absence of a surface glycoprotein with a molecular weight of 115,000 daltons from lymphocytes of three patients. This glycoprotein, now known as sialophorin (CD43), has since been cloned and maps to chromosome 16. As the gene defective in WAS is on the X chromosome, the absence of sialophorin from the cell membrane must be secondary to an underlying abnormality.

In 1980 Gealy et al performed X-inactivation studies in an obligate carrier of WAS who was also heterozygous for the A and B isoenzymes of glucose-6-phosphate dehydrogenase (G6PD). They found that while both isoenzymes were expressed in erythrocytes and neutrophils, only the A isoenzyme was expressed in platelets and T lymphocytes. This experiment was repeated in another obligate carrier female heterozygous for the A and B G6PD isoenzymes. This time only the B isoenzyme was expressed in platelets, granulocytes, monocytes, T lymphocytes, and B lymphocytes. Few women are heterozygous for G6PD polymorphisms, but the recent development of molecular methods for studying X-inactivation patterns has allowed X chromosome use to be investigated in more carriers of WAS. Fearon et al investigated X-inactivation patterns in granulocytes and T and B lymphocytes from eight obligate carrier females and found nonrandom use of the X chromosome in all samples. A further study has confirmed the nonrandom use of the X chromosome in monocytes. As nonrandom use of the X chromosome has been found in all obligate carriers studied, X-inactivation patterns can be used as a carrier test for female relatives of affected males.

WAS was localized to the proximal short arm of the X chromosome by Peacock and Siminovitch. Further linkage studies have identified markers (DXS7 and DXS14) on the short arm of the X chromosome that flank the disease locus. No recombinations have been observed between TIMP, DXS255, DXS146, three markers localized between DXS7 and DXS14, and the disease locus.

The probe M27β detects a variable-number tandem repeat polymorphism at the locus DXS255 and methylation differences between the active and inactive X chromosome. There are no Msp I restriction sites within the repeat motif, the length polymorphism can be seen in Msp I-digested DNA. The recognition site of Msp I (CCCG) and its isoschizomer Hpa II (CCGG) digest short DNA only if the CpG is unmethylated. At least one of the Msp I sites flanking the repeat motif is methylated on all active X chromosomes and unmethylated on most inactive X chromosomes. Thus, in DNA extracted from a sample with nonrandom X-chromosome use, only one of the polymorphic bands will be seen in the HpaII track and this band corresponds to the inactive X chromosome.

We have combined the use of M27β as a linked probe and for methylation studies. This combination has enabled us to say with greater certainty which females are carriers of WAS in families referred for genetic counseling.

MATERIALS AND METHODS

Families. Four of the families referred for genetic counseling are shown in Fig 1. Counseling was requested for all three females in the third generation of family A. The consultand in families B, C, and D is shown by an arrow. In family C there was no previous history of WAS. An additional three mothers of sporadic males were studied. In each family at least one male had been investi-

From the Department of Human Genetics, Newcastle upon Tyne, UK; Hospital General Vall d’Hebron, Barcelona, Spain; MRC Radiobiology Unit, Didcot, Oxon, UK, and the Institute of Child Health, London, UK.

Submitted November 19, 1990; accepted February 6, 1991.

Supported by the Northern Child Health Research Fund (CHILD) and the Scientific and Research Committee of Newcastle Health Authority.

Address reprint requests to Judith Goodship, MD MRCP, Regional Genetics Advisory Service, 19 Claremont Pl, Newcastle upon Tyne NE2 4AA, UK.

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Fig 1. Pedigrees A, B, C, and D are shown. The consultand referred for genetic advice is marked by an arrow in each pedigree. The \textit{Msp} I alleles are numbered from largest to smallest within each family and are not a direct size measurement. The \textit{Msp} I alleles are written alongside each individual. The alleles present in the \textit{Hpa} II track in females are written under the \textit{Msp} I alleles. The presence of two alleles in the \textit{Hpa} II track indicates random use of the X chromosomes and the presence of one allele in the \textit{Hpa} II track indicates nonrandom use of the X chromosome. Allele 3 is segregating with WAS in pedigree A, allele 2 in pedigree B, and allele 5 in pedigree C. Allele 4 is associated with the disease in pedigree D.

\textbf{RESULTS}

A nonrandom pattern of X inactivation was found in the DNA samples from the six obligate carriers in these families.

In family A the two females inheriting the low-risk M27P allele had a random X-inactivation pattern and the female inheriting the high-risk M27P allele had nonrandom use of the X chromosome.

The consultand in family B had a 1 in 12 risk of being a carrier of WAS based on a Bayesian calculation using the pedigree information. Analysis of her DNA showed random use of the X chromosome.

The mother, IIa, of the isolated case in pedigree C, had a nonrandom X-inactivation pattern. Her mother and two sisters also had nonrandom use of the X chromosome. The allele corresponding to the inactive X in IIa was the M27P allele inherited by her affected son. The allele corresponding to the active X in IIa was the allele inherited by her normal son. Similarly, the allele corresponding to the active X in IIa was the allele inherited by her normal son. The two females in generation III in this family inherited the
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Fig 2. Southern blot analysis of DNA from III, and III, in pedigree D, marked 1 and 2 in Fig 1. Track 1 contains Msp I-digested DNA from subject 1, track 2 Hpa II-digested DNA from subject 1, track 3 Msp I-digested DNA from subject 2, and track 4 Hpa II-digested DNA from subject 2. The sisters have the same Msp I fragment sizes. In subject 1 only the lower band is present in the Hpa II track. In subject 2 both polymorphic bands are present in the Hpa II track. The polymorphic bands in the Hpa II tracks are slightly higher than the corresponding bands in the Msp I track because the higher molecular weight in the Hpa II sample affects DNA migration on electrophoresis.

Fig 3. Southern blot analysis of one branch of pedigree A. The DNA in tracks 1 and 2 is from I,, track 3 from I,, tracks 4 and 5 from II,, track 6 from III,, tracks 7 and 8 from III,, track 9 from III,, and track 10 from II,. The DNA in tracks 1, 3, 4, 6, 7, 9, and 10 has been digested with Msp I and the DNA in tracks 2, 5, and 8 has been digested with Hpa II. There is a single band in the Hpa II track of I,, II,, and III,. The band remaining in the Hpa II track, representing the inactive X, is allele 3 in each female. The band present in the two affected males is allele 3.

In the families investigated there were no discrepancies between the carrier status predicted using M27β as a linked probe and the results of the X-inactivation studies, except for family D in which a new mutation was identified.

DISCUSSION

WAS has been mapped to proximal Xp. No recombinations have been reported between the disease locus and DXS255 in 63 informative meioses. Including females whose carrier status has been determined by study of X-inactivation patterns, these families add 15 informative meioses with no recombinations.

Female carriers of WAS have been shown to have a nonrandom X-inactivation pattern in purified granulocytes, monocytes, and lymphocytes using G6PD isoenzymes and DNA probes from the 5’ end of the phosphoglycerate kinase gene (PGK) and the 5’ end of the hypoxanthine-guanine-phosphoribosyl transferase gene (HPRT). These probes detect polymorphisms and methylation differences between the active and inactive X chromosome. We have analyzed DNA extracted from whole blood and confirmed nonrandom use of the X chromosome in the obligate carriers in the pedigrees in Fig 1. Extraction of DNA from venous blood rather than a cell fraction is an advantage if X-inactivation studies are to be used as a routine carrier test in women at high pedigree risk. The probe we have used for studying X-inactivation patterns is M27β. This probe has two major advantages over the PGK and HPRT probes. Firstly, it detects a variable number tandem repeat polymorphism with a reported heterozygosity rate of over 95% and X-inactivation studies can, therefore, be conducted in nearly all females referred. Less than 50% of females in the population we have studied are heterozygous
for the polymorphisms detected at the PGK and HPRT loci. Secondly, M27p is linked to the disease locus.

As the probe that we have used for our X-inactivation studies is closely linked to the disease locus, it is possible to assign phase in families where the pedigree structure is not amenable to conventional use of linked probes. The band remaining in the Hpa II track in a nonrandom sample corresponds to the inactive X chromosome because the Msp I site is methylated on the active X chromosome and unmethylated on most inactive X chromosomes. Thus, in individual I in pedigree A, who has alleles 3 and 5 in the Msp I track and allele 3 in the Hpa II track, allele 3 corresponds to the inactive X, i.e., the X carrying the defective gene (Fig 3). Both carrier daughters, II, and II, have inherited allele 3 and have nonrandom use of the X chromosome. The two affected sons of II, have inherited allele 3. Her daughter, III, has also inherited allele 3 and has nonrandom use of the X chromosome with allele 3 corresponding to her inactive X chromosome (Fig 3).

The DNA analysis of a sporadic male and his mother are shown in Fig 4. The allele remaining in the Hpa II track of the mother corresponds to the X chromosome with the defective gene. This M27p allele has been inherited by her affected son. In pedigree D, although III, has no living male offspring, prenatal diagnosis could be offered using M27p as a linked probe because the defective gene must be on her inactive X chromosome and hence will segregate with allele 4.

X-inactivation studies were particularly useful in the mothers of isolated male cases and in pedigree D. All four mothers of isolated male cases had nonrandom use of the X chromosome. Identification of carriers by and assignment of linkage phase by methylation analysis lead to more precise genetic counseling for female relatives of isolated male cases. In pedigree D we were able to pinpoint where the new mutation occurred, allowing us to reassure other females in the family.

X inactivation is a random event, hence, the percentage of cells with the maternal X chromosome active in a group of normal females will form a normal distribution with a mean of 50. In experiments using neutral G6PD polymorphisms, Fiaklow found a mean of 48, and the range for two standard deviations was 25% to 71%. Similarly, while extreme skewing in the use of the X chromosome has been found using the PGK and HPRT polymorphisms in approximately 5% of women, none of the women had exclusive use of one X chromosome. In the 24 normal females heterozygous for the M27p length polymorphism that we have investigated, two clear bands were visible in the Hpa II track in all cases, even if they were not of equal intensity. However, a normal female with X inactivation at the extreme of the normal distribution would be misclassified as a carrier using this approach. The effect of this can be seen if we consider a false-positive rate of 0.01 in two women, one with a prior risk of 0.5 of being a carrier and the other with a prior risk of 0.01 of being a carrier. In the first woman a nonrandom result alters her carrier probability to 0.99, but in the second woman the same result alters the carrier probability to 0.5. Because of the theoretical false-positive rate using this method we think that carrier testing by study of X-inactivation patterns should be restricted to women with a high prior risk.

When a diagnosis of WAS is made, identification of carrier status in female relatives is important for genetic counseling. There is a small error rate when using linked probes alone for carrier detection due to the possibility of recombination between the marker and disease locus at meiosis. It is not possible to identify new mutations using linked DNA markers. In the families presented here there were no discrepancies between the carrier status predicted using M27p as a linked probe and the results of the X-inactivation studies, except for family D where a new mutation was identified by methylation analysis. Use of M27p for methylation analysis in WAS is more suitable than the PGK or HPRT method as M27p is closely linked to the disease locus. Use of M27p allows assignment of linkage phase by methylation analysis in families that do not have an adequate family structure for tracking a linked probe through the family. In conclusion, we have combined the use of M27p as a linked probe and for methylation studies to determine carrier status for WAS in families referred for genetic counseling.
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