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Mapping the Wilson Disease Locus to a Cluster of Linked Polymorphic Markers on Chromosome 13

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SUMMARY

Linkage of both several chromosome 13 DNA markers and the locus for the red cell enzyme esterase D (*ESD*) to Wilson disease (WD), an autosomal recessive disorder affecting copper metabolism, was investigated in five Middle-Eastern kindreds. The single-copy probe 7D2, identifying the polymorphic region *D13S10*, was demonstrated to lie 7.5 centiMorgans (cM) from the locus, since a maximum lod score of 4.66 at a recombination frequency of .07 (7.5 cM) was found between the locus for WD (*WND*) and *D13S10*. Multipoint linkage analysis between several chromosome 13 markers and *WND* enables us to propose that the order of markers closely linked to *WND* is as follows: centromere–*D13S10–ESD–WND*.

INTRODUCTION

Wilson disease (WD), or hepatolenticular degeneration, is a rare autosomal recessive disorder of copper metabolism characterized by a substantial decrease in biliary excretion of copper. This results in the accumulation of copper in the liver, basal ganglia, and other organs (Danks 1983). Patients present with liver disease and/or an extrapyramidal neurological syndrome in the first or second decade of life. If the disease goes untreated, early death can result from

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liver cirrhosis and paralysis. Variability in clinical symptoms and in age of onset has been observed in patients from different ethnic backgrounds (Cox et al. 1972). The incidence of this disease in Israel among Arabs, Druze, and Oriental Jews is believed to be higher than the incidence of 1 in 40,000 births reported for other Israeli communities (Passwell et al. 1977).

Although WD was recognized at the beginning of this century, the basic biochemical defect of this disorder has not yet been identified. A first step in this direction was evidence for linkage between the locus for WD (*WND*) and that for the red cell enzyme esterase D (*ESD*) on chromosome 13 (Frydman et al. 1985; Bonne-Tamir et al. 1986). The combined maximum lod score ($\hat{Z}$), as based on pooled results from three kindreds, was 5.49 at a maximum-likelihood estimate of recombination frequency ($\hat{\theta}$) of .03.

Until recently only a few useful genetic markers from chromosome 13 were available. Partly because of the localization of hereditary retinoblastoma to chromosome 13 by linkage to *ESD* (Sparkes et al. 1980, 1983), chromosome 13–enriched recombinant-DNA libraries have been constructed and several probes revealing polymorphic loci have been isolated (Cavenee et al. 1984; Dryja et al. 1984; Buys et al. 1985). A genetic map including many of these loci has been developed for the long arm of chromosome 13 (Leppert et al. 1986). Recent experiments have detected the first observed recombinants between *ESD* and *D13S10*, putting *D13S10* proximal to *ESD* (Bowcock et al., in press).

We here report the results of multipoint analysis that have allowed us to predict the position for the WD gene among several chromosome 13 markers in five Israeli families.

MATERIAL AND METHODS

Families Studied

WD had been diagnosed previously in the families studied in the present analysis. These families included a large Israeli-Arab kindred (Frydman et al. 1985), two Druze families (Bonne-Tamir et al. 1986), a Jewish family from Iraq (previously unpublished) in which the parents are first cousins and there is one affected son aged 15 years and two unaffected sibs, and a Jewish family from Iran (unpublished data) in which the parents were first cousins and all three children are affected. As in previous papers that considered some of the families reported here (Frydman et al. 1985; Bonne-Tamir et al. 1986), all relevant laboratory tests and clinical histories were conducted on both affected and normal individuals; moreover, all of the younger unaffected sibs were of an age when abnormal liver function would have been observed were they to develop WD. Considering also their normal copper and ceruloplasmin levels, one can conclude that they were normal.

DNA Analysis

High-molecular-weight DNA was isolated from the buffy coats of family members by means of the technique described by Feder et al. (1985). Three micrograms was digested with the appropriate restriction enzymes and ana-

lyzed by means of Southern blot hybridization (Feder et al. 1985). Probes were labeled according to the method described by Feinberg and Vogelstein (1983). The probes and their source, the enzymes used with each probe to identify a polymorphism, and the locus that they identify are shown in table 1.

Linkage Analysis

Linkage between *WND* and the chromosome 13 markers was investigated with the assistance of the computer programs LIPED (Ott 1974) and LINKAGE (Lathrop et al. 1984). A recessive model with complete penetrance in both sexes and an allele frequency of .005 (Passwell et al. 1977; Scheinberg 1982) were assumed for WD. The results were unaltered over a wide range of allelic frequencies for the mutant allele.

RESULTS

Table 2 shows the lod scores (z values) obtained between *WND* and the chromosome 13 markers. By convention, positive z values $> +3$ represent significant evidence of linkage (Ott 1985, pp. 22–80). There is strong evidence for linkage between *WND* and two marker systems: *D13S10* (interpolated θ values [$\hat{\theta}$] = .07, $\hat{Z}$ = 4.56) and *ESD* ($\hat{\theta}$ = .03, $\hat{Z}$ = 5.20). The 1 lod score–unit support limits (Conneally et al. 1985) for the $\hat{\theta}$ between *WND* and *D13S10* are .01 and .20. An analogous confidence interval with limits of .001 and .15 was previously derived for *WND* and *ESD* (Bonne-Tamir et al. 1986). There is also suggestive evidence for linkage with *D13S4* ($\hat{\theta}$ = .11, $\hat{Z}$ = 2.16) and *D13S6* ($\hat{\theta}$ = .05, $\hat{Z}$ = 1.95). On the basis of two-point analysis alone, there is insufficient evidence for linkage with any other marker.

Multipoint analysis allows one to examine several loci simultaneously (Lathrop et al. 1984) and provides an opportunity to determine the relative position of *WND* among a group of linked markers. It is also more efficient in detecting linkage, because multiple crossovers can be detected and counted. The initial step entailed an approximate localization of *WND* on the fixed map of chromosome 13 (fig. 1) using the program LINKMAP from the LINKAGE package. *D13S3*

TABLE 1

PROBES, ENZYMES USED WITH EACH PROBE TO IDENTIFY A POLYMORPHISM, AND LOCUS THAT EACH IDENTIFIES

Probe (Source)	Enzyme	Locus
p7F12 (W. Cavenee)	*Msp*I/*Taq*I[a]	*D13S1*
p9D11 (W. Cavenee)	*Msp*I/*Taq*I[a]	*D13S2*
p9A7 (W. Cavenee)	*Msp*I/*Hin*dIII[a]	*D13S3*
p1E8 (W. Cavenee)	*Msp*I	*D13S4*
pHUB8 (T. P. Dryja)	*Eco*RI/*Hin*dIII[a]	*D13S5*
pHU10 (T. P. Dryja)	*Xmn*I	*D13S6*
pHU26 (T. P. Dryja)	*Bgl*II	*D13S7*
7D2 (M. Leppert)	*Taq*I/*Dra*I[a]	*D13S10*

[a] Treated as haplotypes.

TABLE 2

RESULTS OF TWO-POINT LINKAGE ANALYSIS BETWEEN WD AND CHROMOSOME 13 MARKERS

MARKER	RECOMBINATION FRACTION (θ)								
	.00	.001	.05	.10	.20	.30	.40	$\hat{\theta}^a$	$\hat{Z}$
D13S1	$-\infty$	−6.85	−1.05	−0.02	0.50	0.39	0.11	.22	0.52
D13S2	$-\infty$	−7.16	−1.28	−0.11	0.59	0.47	0.15	.22	0.67
D13S3	$-\infty$	−12.68	−3.91	−2.25	−0.83	−0.23	−0.09	.5	0
D13S4	$-\infty$	−4.53	1.61	2.14	1.85	1.12	0.40	.11	2.16
D13S5	$-\infty$	−4.72	−0.92	−0.28	0.10	0.11	0.01	.25	0.13
D13S6	0.82	1.04	1.95	1.79	1.12	0.66	0.20	.05	1.95
D13S7	0.14	0.14	0.12	0.10	0.05	0.03	0.01	0	0.14
D13S10	$-\infty$	2.43	4.46	4.48	3.54	2.24	0.82	.07	4.56
ESD	$-\infty$	4.00	5.16	4.78	3.67	2.40	1.10	.03	5.20

[a] Value at which $\hat{Z}$ is greatest.

was excluded from the analysis because it is ≥45 centiMorgans (cM) from *ESD* (fig. 1). *D13S4* and *D13S5* were combined into a single haplotype system, and *D13S7* was uninformative and therefore excluded. Thus, a total of six distinct loci, in addition to the disease locus, were considered in the multipoint analysis. In our analyses we did not vary the order and distances between the markers. We had previously calculated a distance of 5.5 cM between *D13S10* and *ESD* (Bowcock et al., in press). Distances between all other markers were taken from Leppert et al. (1986).

A seven-point analysis was not practical, and thus a series of overlapping three- and four-point analyses (Drayna and White 1985) was carried out to determine the most likely position of the *WND* among the nearest six marker loci. Possible orders are indicated by a number corresponding to the position of *WND* in figure 1. The two most favored orders are *D13S6–D13S1–D13S10–WND–ESD–D13S2–D13S4/D13S5* (order 4) and *D13S6–D13S1–D13S10–ESD–WND–D13S2–D13S4/D13S5* (order 5), with odds of 45:1 in favor of order 5 over order 4. This support was derived primarily from two offspring in these families who exhibit an obligate crossover between either *ESD* or *D13S10* and *WND* (fig. 2). Our interpretation regarding the position of *WND* with respect to *ESD* and *D13S10*, as based on the program LINKMAP, is diagrammed in figure 2. Parental haplotypes shown in this figure represent a most parsimonious inference of phase, as based on examination of haplotypes in three affected offspring (fig. 2), eight unaffected offspring (table 3), and two sibs of each parent (data not shown). The reader should note that the spouses of these maternal sibs are sibs of each other and that the parents in figure 2 are half–first cousins (Frydman et al. 1985). These familial relationships contributed to the inference of phase in this figure.

Relative to order 5, orders 2 and 6 are excluded by odds of 3×10^{10}:1 and 9×10^{14}:1, respectively, orders 1 and 7 are excluded by odds of >10,000:1, and order 3 is excluded by odds of 6,339:1. The $\hat{\theta}$ values between *WND* and its probable flanking markers *ESD* and *D13S2* are .03 and .15, respectively. For

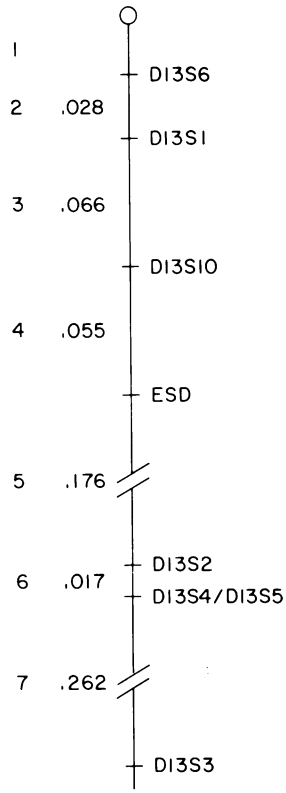

FIG. 1.—Genetic map of the long arm of chromosome 13. $\theta_m = \theta_f$ is assumed. Decimals to the left of the line represent previously reported recombination frequencies between each pair of loci (Leppert et al. 1986), with the exception of the distance between *ESD* and *D13S10*, which was derived by us (Bowcock et al. 1987) (distances not drawn to scale). Evidence in support of this order was confirmed by three-point linkage analysis of the genotype data in our WD families (data not presented). The overall finding of twice as much recombination in females as in males in the Utah families was verified in our data by means of quadratic interpolation with the computer program QUAD2 (Pakstis et al. 1986). Integers to the far left of the line denote possible locations for *WND* with respect to these seven loci.

order 5, based on a 95% confidence interval for θ, *WND* is 1–12 cM on the distal side of *ESD*. The *WND–ESD* distance was not affected by significantly increasing or decreasing distances between any marker loci and is based on the assumption of equal recombination rates in males and females. A higher recombination frequency in females has been observed for loci on chromosome 13 (Leppert et al. 1986). This is also evident in the families in the present study (table 3). However, separate analysis allowing for increased recombination in females did not significantly alter the support favoring order 5 over any of the other orders.

DISCUSSION

Using multipoint linkage analysis, we have been able to demonstrate that *WND* is likely to be located distal to *ESD*, at a tentative distance of 3 recombi-

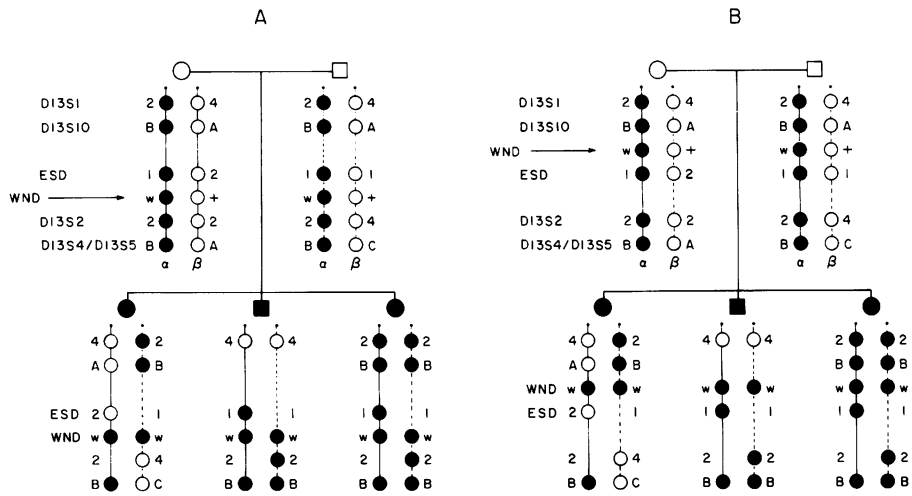

FIG. 2.—Alternative orders for *WND* with respect to *ESD* and *D13S10*, in the only pedigree in which crossing-over was detected between the disease locus and these markers. Only the three affected offspring are shown (the parental origin of chromosome 13 homologues for the eight unaffected sibs are shown in table 3). *WND* is proximal to *ESD* in *A* and distal in *B*. *A* was deduced by LINKMAP to be 45 times more likely than *B*. Where it could be derived, the phase of the alleles (chromosome 13 DNA markers, esterase D alleles, and WD alleles [w and +]) is shown. Each allele is shown as a circle on a homologous chromosome diagrammed under the pedigree symbol. Alleles from parental WD chromosomes (alpha) are shown as solid circles. Alleles from the parental non-WD chromosomes (beta) are shown as open circles. *A* requires four single crossover events. *B* requires one triple and three single crossover events but could also be explained by two double crossovers in child 1.

nation units with a 95% confidence interval extending from 1 to 12 recombination units. These conclusions are predicted on the assumed map distances in figure 1, which are subject to sampling error (Leppert et al. 1986; Bowcock et al., in press). Considering the prior estimated distance between *WND* and *ESD* (0.03; Bonne-Tamir et al. 1986), one might think it extraordinary if the map order for *WND* were any other than order 4 or order 5 (see fig. 1). However, when based on results from two-point linkage analysis, judgments about map order for closely linked loci may be erroneous (consider, e.g., that the order of loci on the short arm of chromosome 11 is controversial [Kramer et al. 1986; Meyers et al. 1986]). Given the rather large confidence interval between *WND* and *ESD* (Bonne-Tamir et al. 1986), prior to this analysis it was plausible that *WND* could be located in any position between the centromere and *D13S2*—i.e., orders 1–5 could have been correct; however, all but two of these orders were significantly ruled out by multipoint linkage analysis.

At the moment the closest (at 3 cM) marker to *WND* is still *ESD*, but it has a low polymorphism information content (PIC) (Botstein et al. 1980) of 0.16. The closest DNA marker presently available is *D13S10*. It is a little farther away but now has a PIC of 0.35 with *Dra*I (Bowcock et al., in press) and 0.09 with *Taq*I (Willard et al. 1985). Thus, in fine-structure mapping studies and in the molecular diagnosis of WD in other families, *D13S10* will be more useful than the

TABLE 3

TRANSMISSION OF CHROMOSOME 13 HOMOLOGUES TO UNAFFECTED OFFSPRING IN THE SIBSHIP DEMONSTRATING CROSSOVERS BETWEEN *WND* AND *ESD* OR *D13S10*

Child (Sex)	Maternal Chromosome	Paternal Chromosome
1 (M)	Beta	Alpha
2 (F)	Alpha-D13S1:D13S10-beta	Alpha
3 (M):[a]		
a	Beta	Alpha-D13S1:D13S10-beta
b	Alpha-D13S1:D13S10-beta	Beta
4 (F)	Alpha	Beta
5 (F)	Beta-ESD:D13S4/D13S5-alpha	Beta
6 (M)	Beta	Alpha
7 (M)	Beta	Beta
8 (M)	Beta	Alpha-ESD:D13S2-beta

NOTE.—See fig. 2 for description of parental chromosomes alpha and beta and transmission of chromosome 13 homologue to the three affected offspring (not included in this table). Colon (:) indicates obligate crossovers between loci on either side.

[a] Genotypes a and b are indistinguishable alternatives that are equally likely if equal θ values are assumed for males and females. Given previously published results (Leppert et al. 1986) and our data (fig. 2), a maternal crossover is more likely, thereby favoring genotype b.

serum protein polymorphism for esterase D. Moreover, prenatal testing for WD by means of esterase D requires invasive sampling of fetal blood, which may be contaminated by maternal serum. Identification of polymorphic probes between *D13S10* and *D13S2* will be necessary to map *WND* more definitively.

Multipoint linkage analysis has facilitated exclusion of several inherited diseases (e.g., cystic fibrosis [Wainwright et al. 1986], multiple endocrine neoplasia type 2A [Farrer et al., in press], and torsion dystonia [Kramer et al., in press]) from large chromosomal segments. It has also been used to establish linkage between cystic fibrosis and two linked genetic markers (Tsui et al. 1985). In the present report multipoint linkage analysis of families has enabled us to predict the position of *WND* on chromosome 13. In addition, we have been able to distinguish heterozygotes from normal homozygotes with a confidence level significantly higher than that possible under Mendelian expectations (Farrer et al., submitted).

One question that needs to be addressed regarding linkage between markers and a disease locus is that of genetic heterogeneity. If a disease is due to mutations at a number of unlinked loci, preclinical and prenatal diagnosis that uses genetic markers shown to be closely linked in some families is not reliable. Although there is in the literature (see, e.g., Cox et al. 1972) an indication of clinical heterogeneity for WD, this need not be determined by different loci. We studied five independently ascertained families, and all appear to show linkage to one locus on chromosome 13. The maximum proportion of families with the WD mutation located at other loci can be calculated by means of simple binomial theory (Sokal and Rohlf 1969, pp. 71–81), and the appropriate equation is $y = x^n$, where y = a specified confidence level, x = the proportion of linked families, and n = the number of families. Thus, on the basis of data

from five linked families, at the 5% level we can conclude that other loci do not account for WD in >45% of Middle-Eastern families. Application to prenatal and preclinical diagnosis in non-Middle-Eastern families should await information on more families from a wider geographical origin.

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