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Assignment of the gene for Wilson disease to chromosome 13: Linkage to the esterase D locus

(gene mapping/linkage of autosomal recessive diseases/copper metabolism)

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ABSTRACT **Wilson disease (WD) is an autosomal recessively inherited disorder of copper metabolism for which the basic defect is still unknown. Twenty-seven autosomal markers were investigated for linkage in a large inbred kindred with affected individuals in two generations. Also, serum copper and ceruloplasmin were measured on all available members. Close linkage ($\theta = 0.06$) with a logarithm of odds (lod) score of 3.21 was found between the gene for WD and the esterase D locus. Efficient detection of linkage was made possible by the use of a multisibship inbred pedigree. The discovery of a polymorphic marker genetically linked to the WD locus has profound implications both for investigation of the primary gene defect and for clinical services.**

Wilson disease (WD), or hepatolenticular degeneration, is characterized by gross reduction in the incorporation of copper into ceruloplasmin and a substantial decrease in biliary excretion of copper; both are associated with accumulation of copper in the liver, basal ganglia, and other organs, causing tissue damage and death, if untreated (1). In addition to the typical clinical features the diagnosis is based on documentation of low serum ceruloplasmin, decreased plasma copper, and increased urinary copper excretion. Increased liver copper concentration and lack of incorporation of ^{64}Cu into ceruloplasmin are considered the most reliable tests (1).

Autosomal recessive inheritance is well established throughout the world (2–6), but because of the variability in liver pathology, neurological symptoms, and age of onset, genetic heterogeneity has been proposed (5, 6). The primary abnormality in WD, a disorder recognized since the beginning of this century and intensively studied over the last 30 years, has not been identified.

Three individuals affected with WD, each in a different branch of a large inbred Israeli–Arab lineage, were independently ascertained (Fig. 1). Two of them (IV-7 and IV-19) presented neurological symptoms, and the third (IV-33) had liver disease.

All living individuals in the pedigree were examined and serum copper (using atomic absorption at λ 324.7 nm, air acetylene flame, and visimax hollow cathode lamp; IL video II instrument) ceruloplasmin (radial immunodiffusion, Norpartigen; Behring, Somerville, NJ), and several liver enzyme levels were measured. In individuals with low ceruloplasmin or abnormal liver enzyme levels, 24-hr urinary excretion of copper was determined. Individual IV-7 had a liver biopsy and WD was confirmed by increased liver copper content.

Ninety-four acid citrate dextrose anticoagulated blood specimens from members of the kindred including eight affected individuals and eight obligatory heterozygotes were investigated for the following genetic markers: (*i*) erythrocyte antigens: ABO, Rh, MNS, Kell, Duffy, P, Kidd, and Lutheran; (*ii*) erythrocyte enzymes: adenylate kinase, adenosine deaminase, esterase D, phosphoglucomutase 1 and 2, glutamate pyruvate transaminase, glyoxalase 1, peptidase A and B, phosphohexose isomerase, 6-phosphogluconate dehydrogenase, acid phosphatase, lactic dehydrogenase, malic dehydrogenase, and phosphoglycolate phosphatase; (*iii*) plasma protein: haptoglobin and transferrin.

Typing of the erythrocyte antigens, preparation of hemolysates, and tests for electrophoretic variants of the enzymes and serum systems are described elsewhere (7). All affected individuals, their parents, and selected normal sibs were also tested for ABH blood group substances in their saliva; taste sensitivity to phenylthiocarbamide; and HLA-A, -B, -C, and -D antigens by the standard two-stage microcytotoxicity test (courtesy of R. Zamir).

Lod scores for linkage and their maximum likelihood estimates were calculated using the computer program LIPED, version 3 (8). This version of LIPED, based on the algorithm of Lange and Elston (9), was designed to accommodate complex family structures (e.g., consanguinity). Lange and Elston consider a pedigree with "cycles" or consanguinity loops as a specialized case of a complex pedigree. Five cycles were broken by inserting in this pedigree a genetic and phenotypic copy (i.e., "doubled" individual) next to one of the marriage partners in each cycle. This procedure results in the creation of several linked pedigrees to replace the original pedigree. Doubled individuals are iteratively clipped from the pedigree in the likelihood calculation at later stages in the algorithm. Hence, the likelihood represents a sum of products of likelihoods over all simple pedigrees that contain a doubled individual.

The pedigree shown in Fig. 1, indicating WD and esterase D phenotypes, is consistent with autosomal recessive inheritance of WD. The study disclosed five presymptomatic individuals, including one in a family not previously known to segregate for the abnormal gene. Although an excess of affected females was found (7:1), the two males reported to have died in childhood of a liver disease may have been afflicted with WD.

Segregation was found for 15 autosomal markers. Logarithm of odds (lod) scores for these marker systems are given in Table 1. In this table, it is assumed that the male and female recombination fractions are equal. With the exception of esterase D there is no evidence of linkage with any of these markers. Interpolation between $\theta = 0.00$ and $\theta = 0.10$ yielded the maximum likelihood estimate of recombination between WD and the esterase D locus, which is 0.06 with a lod score of 3.21.

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Abbreviation: WD, Wilson disease.

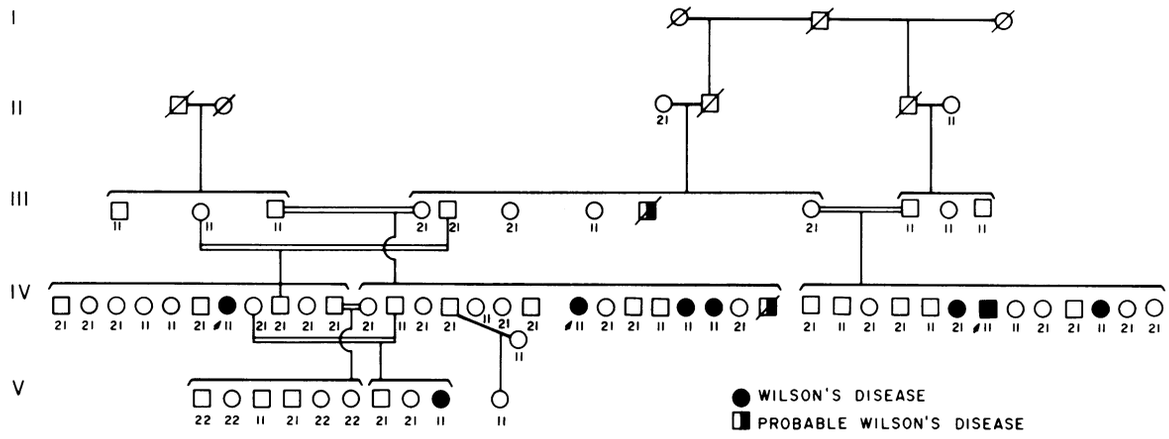

FIG. 1. Pedigree of an Israeli–Arab family with WD. Circles, females; squares, males; slashed symbol, deceased; affected and probably affected individuals with WD are indicated according to the key in the diagram. A double line connecting two individuals denotes a consanguineous union. Probands are identified by an arrow. Phenotypes at the esterase D locus are shown under each symbol.

In this family, the gene for WD is segregating with the one allele of esterase D. The only instance of a documented crossover between these two loci occurred in one of the affected offspring who is 2:1 for esterase D. Because homozygous normal and heterozygous individuals are phenotypically identical with respect to WD, there is a possibility for undetected crossover events among those in this group.

Historically, there have been few successful attempts to link autosomal recessive disorders by classical family study methods. Families in which such traits segregate are usually small, contain few affected individuals, and lack information about carrier status among phenotypically normal individuals. Thus, in linkage analysis of autosomal recessive disorders the magnitude of the lod score is primarily determined by the pedigree structure. A family with a number of affected sibships (usually as a result of consanguinity) is much more efficient in gene mapping than a series of unrelated sibships.

Esterase D is a polymorphic genetic marker that was initially assigned to chromosome 13 by Van Heyningen *et al.* (10) and confirmed by Chen *et al.* (11) using somatic cell hybridization techniques. Robson *et al.* (12) studied Robertsonian translocations involving chromosome 13 and localized esterase D to the distal half of the q arm. More recent studies of gene expression of esterase D in patients with retinoblastoma and deletions of chromosome 13 (13–15) have demonstrated that esterase D and the locus for predisposition to retinoblastoma map to the same 11 sub-band of the 13q14 region.

The recombination fraction of 6 centimorgans obtained in this study suggests that the esterase D locus and the gene for WD are closely linked. Because the data in this study were obtained from only one family, further study is required to confirm these linkage results. Confirmation of linkage between esterase D and WD and more precise localization will likely be performed using a library of chromosome 13 restriction fragment length polymorphisms. DNA polymorphic loci have already been identified in this region (16, 17).

Upon confirmation of the linkage, it should be feasible to attempt the cloning and characterization of the abnormal gene on the basis of its map location. Understanding the gene defect may help unravel the mysteries of clinical and age-at-onset heterogeneity (5, 6) and ultimately lead to improved treatments. Cox *et al.* (6) have suggested the existence of at least three types of WD, distinct by their age of onset, clinical symptoms, and biochemical parameters. Although the age of onset of the disease symptoms was fairly uniform in this family, the males presented with liver disease, while the females presented with neurological manifestations. Cultural and environmental factors such as cooking

Table 1. lod scores for linkage between WD and several blood group markers

		Recombination fraction					
Chromosome	Locus	0.00	0.05	0.10	0.20	0.30	0.40
1	Rh	$-\infty$	−1.33	−0.34	0.25	0.34	0.23
	Fy	$-\infty$	−2.57	−1.08	−0.02	0.22	0.16
	PGM1	$-\infty$	0.43	0.53	0.49	0.35	0.18
2	Kidd	$-\infty$	−2.98	−2.10	−1.21	−0.66	−0.26
	ACP	0.76	0.70	0.64	0.47	0.28	0.09
4	MNS	$-\infty$	−1.33	−0.34	0.25	0.34	0.23
6	Glo	$-\infty$	−0.18	−0.01	0.00	−0.04	−0.02
	P	0.89	0.85	0.76	0.53	0.25	−0.01
9	ABO	−0.88	0.18	0.34	0.36	0.23	0.10
	AK	$-\infty$	−1.36	−1.08	−0.77	−0.50	−0.23
13	EsD	$-\infty$	3.21	3.11	2.56	1.79	0.90
16	Hp	0.38	0.34	0.29	0.19	0.10	0.04
	GPT	$-\infty$	−2.18	−1.22	−0.42	−0.10	0.01
20	ADA	$-\infty$	−1.05	−0.53	−0.11	0.05	0.07
Unassigned	Kell	−3.15	−0.05	0.18	0.32	0.30	0.19

PGM1, phosphoglucomutase 1; ACP, acid phosphatase; Glo, glyoxalase 1; AK, adenylate kinase; EsD, esterase D; Hp, haptoglobin; GPT, glutamate pyruvate transaminase; ADA, adenosine deaminase.

in copper vessels, dietary habits, or viral liver disease may add to the variability in clinical expression (1). Our finding of linkage between WD and a polymorphic marker may thus help to determine whether the phenotypic variability is due to allelic or nonallelic heterogeneity or to environmental factors.

Although recent studies (18, 19) have reported increased copper levels in cultured fibroblasts of patients with WD, the observation has not been sufficiently established (1), and the applicability of this method for prenatal diagnosis of WD has not yet been tried (20). It is interesting to note that the gene for the copper-containing protein ceruloplasmin appears to be located on chromosome 3 (21, 22).

Consanguineous marriages are relatively common among Middle Eastern and Arab communities, where the prevalence of WD is considerably higher than elsewhere. This linkage thus potentiates presymptomatic and prenatal diagnosis among those families in which both parents are heterozygous for esterase D and have had at least one affected child. These conditions are necessary to establish linkage phase or the segregation pattern among the esterase D alleles with the mutant gene. The development of DNA markers closely linked on chromosome 13 will enhance the likelihood of an informative test.

Note Added in Proof. We have recently typed an unrelated 10-member sibship with Wilson disease for esterase D. The maximum lod score was 1.48 at $\theta = 0$. The combined maximum lod score was 4.55 at $\theta = 0.04$.

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1. Danks, D. M. (1983) in *Metabolic Basis of Inherited Disease*, ed. Stanbury, J. B. (McGraw-Hill, New York), pp. 1251–1268.
2. Bearn, A. G. (1960) *Ann. Hum. Genet.* **24,** 33–43.
3. Arima, M. & Saro, I. (1968) *Birth Defects Orig. Artic. Ser.* **4,** 54–59.
4. Strickland, G. T. (1973) *Quart. J. Med.* **42,** 619–638.
5. Paswell, J., Adam, A., Garfinkel, D., Streiffler, M. & Cohen, B. E. (1977) *Isr. J. Med. Sci.* **13,** 15–19.
6. Cox, D. W., Fraser, F. C. & Sass-Korstak, A. (1972) *Am. J. Hum. Genet.* **24,** 646–666.
7. Bonné-Tamir, B., Ashbel, S. & Bar-Shani, S. (1978) *Am. J. Phys. Anthropol.* **49,** 457–464.
8. Ott, J. (1977) *Clin. Genet.* **12,** 119–124.
9. Lange, K. & Elston, R. C. (1975) *Hum. Hered.* **25,** 95–105.
10. Van Heyningen, V., Bobrow, M., Bodmer, W. F., Gardiner, S. E., Povey, S. & Hopkinson, D. A. (1974) *Ann. Hum. Genet.* **38,** 295–303.
11. Chen, S., Creagan, R. P., Nichols, E. A. & Ruddle, F. H. (1975) *Birth Defects Orig. Artic. Ser.* **11,** 99–102.
12. Robson, E. B., Hopkinson, D. A., Buckton, K. E., Robinson, J. & Polani, P. E. (1976) *Birth Defects Orig. Artic. Ser.* **12,** 351–354.
13. Sparkes, R. S., Sparkes, M. C., Wilson, M. G., Towner, J. W., Benedict, W., Murphree, A. L. & Yunis, J. J. (1980) *Science* **208,** 1042–1044.
14. Sparkes, R. S., Murphree, A. L., Lingua, R. W., Sparkes, M. C., Field, L. L., Funderburk, S. J. & Benedict, W. F. (1983) *Science* **219,** 971–975.
15. Ward, P., Packman, S., Loughman, W., Sparkes, M., Sparkes, R., McMahon, A., Gregory, T. & Ablin, A. (1984) *J. Med. Genet.* **21,** 92–95.
16. Cavenee, W., Leach, R., Mohandas, T., Pearson, P. & White, R. (1984) *Am. J. Hum. Genet.* **36,** 10–24.
17. Dryja, T. P., Rapaport, J. M., Weichselbaum, R. & Bruns, G. A. P. (1984) *Hum. Genet.* **65,** 320–324.
18. Chan, W. Y., Cushing, W., Coffman, M. A. & Rennert, O. M. (1980) *Science* **208,** 299–300.
19. Camakaris, J., Ackland, L. & Danks, D. M. (1980) *J. Inherit. Metab. Dis.* **3,** 155–157.
20. Chan, W. Y. & Rennert, O. M. (1982) *Ann. Clin. Lab. Sci.* **12,** 372–380.
21. McKusick, V. A. & Conneally, P. M. (1984) *Birth Defects Orig. Artic. Ser.* **20,** 205–209.
22. Weitkamp, L. R. (1984) *Birth Defects Orig. Artic. Ser.* **20,** 604–605.