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Localization of a Gene for Molybdenum Cofactor Deficiency, on the Short Arm of Chromosome 6, by Homozygosity Mapping

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Summary

Molybdenum cofactor deficiency (MoCoD) is a fatal disorder manifesting, shortly after birth, with profound neurological abnormalities, mental retardation, and severe seizures unresponsive to any therapy. The disease is a monogenic, autosomal recessive disorder, and the existence of at least two complementation groups suggests genetic heterogeneity. In humans, MoCoD leads to the combined deficient activities of sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. By using homozygosity mapping and two consanguineous affected kindreds of Israeli-Arab origin, including five patients, we demonstrated linkage of a MoCoD gene to an 8-cM region on chromosome 6p21.3, between markers D6S1641 and D6S1672. Linkage analysis generated the highest combined LOD-score value, 3.6, at a recombination fraction of 0, with marker D6S1575. These results now can be used to perform prenatal diagnosis with microsatellite markers. They also provide the only tool for carrier detection of this fatal disorder.

Introduction

Molybdenum cofactor deficiency (MoCoD; MIM 252150 and 252160) is a fatal inherited disorder manifesting shortly after birth and resulting from the absence of a molybdenum-complexed pterin cofactor (Johnson et al. 1980). The most noted clinical features are neonatal seizures unresponsive to any therapy; abnormal tone and opisthotonos; mental retardation; cranofacial dysmorphic features, including narrow bifrontal diameter and enophthalmos; ectopia lentis; and neurological deterioration (Slot et al. 1993). No treatment has been successful in halting or reversing the progression of neurological damage (Johnson and Wadman 1995). Biochemically, MoCoD is caused by defects in the biosynthesis of the molybdenum-complexed pterin that is the cofactor for three enzymes: sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. Sulfate oxidase deficiency causes accumulation of sulfite, taurine, S-sulfocysteine, and thiosulfate in the urine, and deficiency of xanthine dehydrogenase causes xanthinuria and hypouricemia. The pathogenesis of the clinical features is not understood, but, because this clinical pattern is similar to that seen in isolated sulfite oxidase deficiency, the pathogenesis has been assumed to be related to an accumulation of sulfite metabolites or to a need, by the developing brain, for this enzyme or its product (Johnson and Wadman 1995). Prenatal diagnosis is determined by demonstration of either sulfite oxidase deficiency in chorionic villus direct preparations or increased S-sulfocysteine in amniotic fluid (Gray et al. 1990; Johnson et al. 1991*b*). Postnatal diagnosis is suggested by hypouricemia and the finding of elevated urinary metabolites, as described above (van Gennip 1987), and is confirmed by demonstration of sulfite oxidase deficiency in fibroblasts.

The disease is a monogenic, autosomal recessive disorder. The existence of complementation groups type A (MIM 252150) and type B (MIM 252160), observed in patients' fibroblasts (Johnson et al. 1989), suggests the possibility of multiple genetic variants of MoCoD.

Thus far, MoCoD has been reported to be present in >50 families belonging to various ethnic groups (Arnold et al. 1993; Johnson et al. 1993; van Gennip et al. 1994; Mize et al. 1995; Bonioli et al. 1996; Yurdakok and

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Coskun 1997). For this study, we conducted a full genome search, using polymorphic microsatellite markers in two MoCoD-affected inbred kindreds of Arab-Israeli origin. One of these kindreds was very large and included eight subfamilies. Using homozygosity mapping, we found genetic linkage between MoCoD and several markers in an 8-cM region on chromosome 6p21.3.

Subjects and Methods

Families and DNA Preparation

Two unrelated Arab-Israeli consanguineous MoCoD kindreds were included in the study. The pedigrees of the two kindreds are shown in figure 1. These families live in two close villages in northern Israel. Kindred 1 consisted of eight subfamilies, with a total of 18 affected children. Among the 18 affected children, we had access to DNA samples from 4 patients (5102, 5104, 6101, and 5140). In this kindred, the first MoCoD patient (5140) was diagnosed in 1993 (van Gennip et al. 1994). The construction of a detailed pedigree of kindred 1 disclosed 14 additional patients (including several siblings of MoCoD patients), who had died undiagnosed during the 5 years prior to the study. A review of the medical files and autopsy reports strongly suggested that these infants suffered from MoCoD. Note that, since the first MoCoD patient was diagnosed, three additional affected babies (5104, 5102, and 6101) from this kindred were born to young first-cousin parents. Kindred 2 consisted of two deceased patients (64 and 65). The first patient died undiagnosed, but her medical history supported diagnosis of MoCoD. The second patient (65) was born and diagnosed in 1994. We only had access to a DNA sample from patient 65. In the two families, the diagnosis of MoCoD was based on clinical symptoms that appeared shortly after birth (mainly, neonatal seizures unresponsive to any therapy, opisthotonos, and facial dysmorphism), a biochemical finding of hypouricemia and abnormal sulfur and purine metabolites in urine, and the detection of sulfite oxidase deficiency in fibroblasts. The clinical features of patients of both families were similar, except for the hypertonicity and opisthotonos, which were more pronounced in the patients from kindred 2. Obligate-heterozygous parents did not display any symptoms. EDTA blood samples for genetic analysis were collected from each living family member, after informed consent was obtained from the subjects or their guardians, in accordance with the guidelines of the local institutions. DNA was obtained from fibroblast cell lines, for patients 5140, 5104, and 65, and from EDTA blood samples, for patients 6102 and 5102. DNA was prepared by standard methods (Sambrook et al. 1989). Altogether we had access to DNA samples from 5 patients and 24 healthy subjects.

Genotype Analysis

A genomewide screen was performed on an automated sequencing machine by use of a panel of 240 highly polymorphic microsatellite markers (CA), spaced at ~10-cM intervals, chosen from the Généthon panel (Dib et al. 1996). Extended haplotypes were constructed by use of dinucleotide-repeat markers (CA) in the candidate region and the sex-averaged genetic map described by Généthon (Dib et al. 1996), as shown in figure 2. Genotyping of the markers was performed, by use of a nonradioactive-labeling procedure, with the ECL system (Amersham), on the basis of a horseradish peroxidase–mediated chemiluminescent reaction, in accordance with the study by Gyapay et al. (1996).

Statistical Analysis

Two-point linkage analysis of the disease locus and the genetic markers was performed by use of the MLINK program from the FASTLINK optimized version of the LINKAGE package (Lathrop et al. 1984; Cottingham et al. 1993; Schaffer et al. 1994). An autosomal recessive model with complete penetrance for both sexes and a frequency of .001 for the disease allele was assumed. Since the allele frequencies of the markers were not known, for the particular population group of the study, LOD scores were calculated by assumption of equal allele frequencies. Similar results were obtained when population allele frequencies were estimated from the data (results not shown).

Results

Primary Mapping of the MoCoD Locus in the Israeli-Arab Kindreds

A genomewide screen of the two MoCoD-affected kindreds of Israeli-Arab origin (kindreds 1 and 2; fig. 1) was performed. Two markers, D6S291 and D6S282, on chromosome 6 gave positive LOD scores, suggesting linkage between MoCoD and chromosome 6p, in these two families. A panel of 13 additional markers were genotyped in this region. Two-point linkage analysis was performed. Evidence for linkage to this region was found in two kindreds. Positive LOD scores were obtained for all markers tested. Marker D6S1575 gave the highest combined LOD-score value, 3.6, at a recombination fraction of 0. These data confirmed linkage of the MoCoD locus to chromosome 6p in the Israeli-Arab families.

Haplotype Analysis and Recombination Events in the Israeli-Arab Kindreds

Extended haplotypes were constructed, by use of the 15 microsatellite markers, for all subjects from consan-

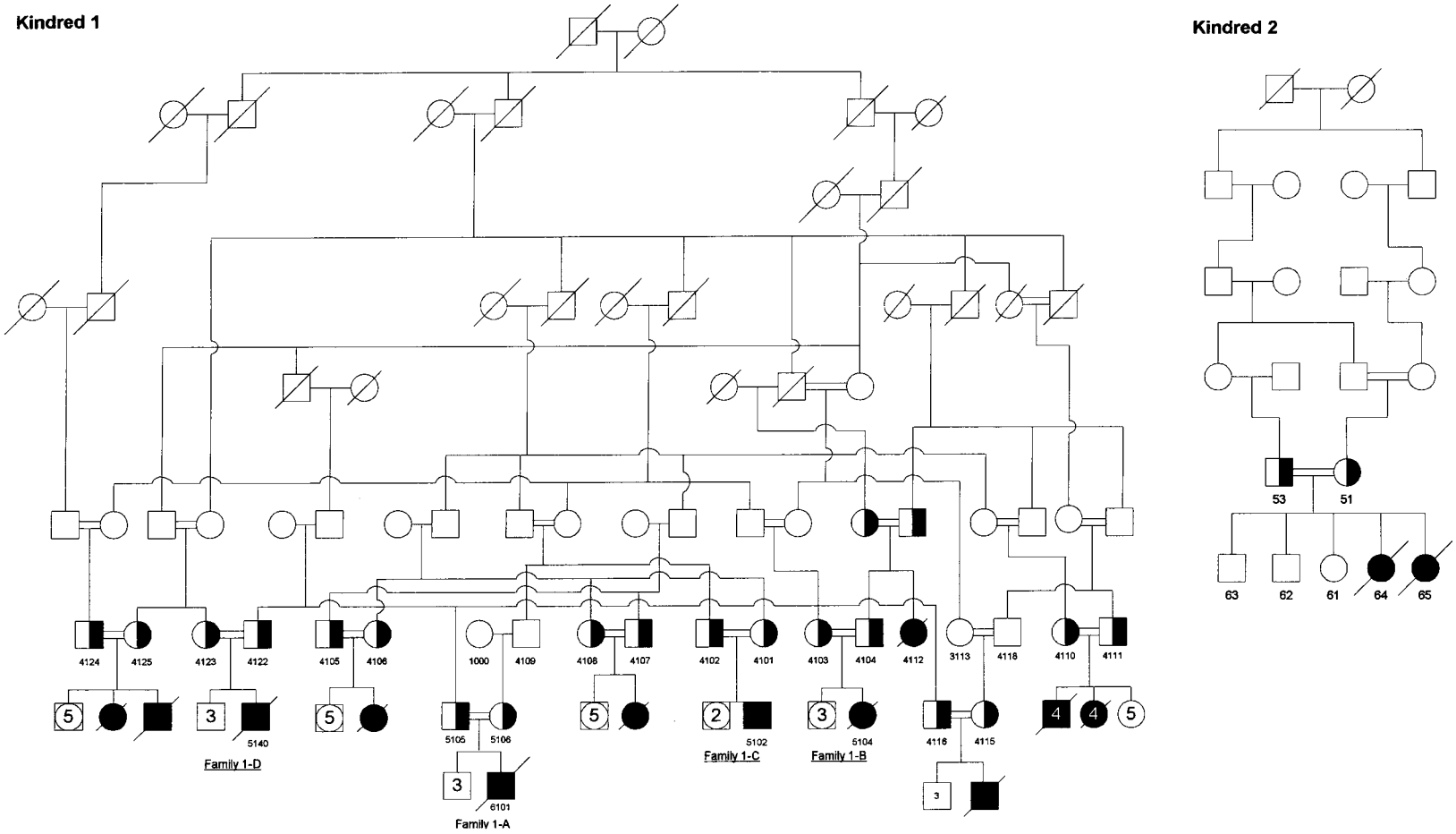

Figure 1 Pedigrees of the two MoCoD kindreds. Kindreds 1 and 2 were consanguineous. Blood samples (or fibroblast cell lines [see Subjects and Methods]) and genotypes were obtained from all numbered individuals. Numbers within a circle or a square correspond to the number of siblings. Altogether we had access to DNA samples from 5 patients and 24 healthy subjects.

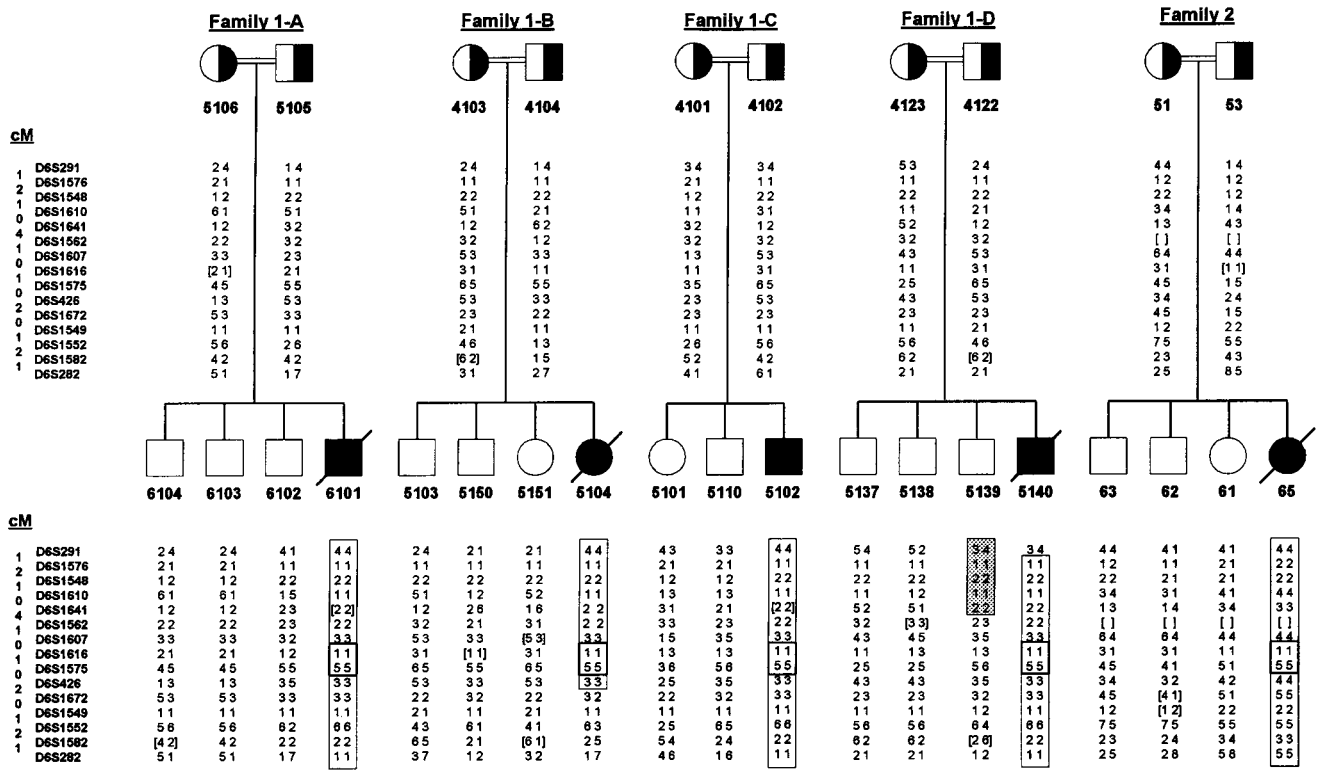

Figure 2 Haplotype analysis, on chromosome 6p21, of MoCoD kindreds 1 and 2. Only key subjects are shown. Telomeric to centromeric markers are arranged from top to bottom. The linked haplotype is boxed. The shaded area corresponds to segments of haplotypes shared between healthy individual 5139 and affected individual 5140. Alleles in parenthesis were inferred. In family 2, marker D6S1562 was technically unsatisfactory and is not shown.

guineous kindreds 1 and 2. Results are shown in figure 2. As was expected, on the basis of homozygosity by descent, regions of homozygosity were observed in the genomes of all affected individuals. Each of the five subjects with MoCoD was homozygous for all loci between and including D6S1576 and D6S426. Flanking this region of consistent homozygosity, a heterozygous genotype was found at marker D6S1672 for the affected individual (5104) in subfamily B of kindred 1, thus defining the centromeric limit of the MoCoD gene interval at this marker. Similarly, the affected individual (5140) in subfamily D of kindred 1 was found to be heterozygous at marker D6S291, thus defining the telomeric limit of the disease gene interval at this marker. We were able to replace this limit by the marker D6S1641. Comparison of the haplotypes of affected individual 5140 with the haplotypes of healthy sibling 5139, both from subfamily D of kindred 1, showed one homozygous segment of shared haplotypes, between D6S291 and D6S1641, and one segment (D6S1562–D6S282) in which the haplotypes differed as a result of a recombination event on the paternal chromosome of the healthy individual. This observation enabled us to exclude from the MoCoD gene interval the region above marker D6S1641. Thus, we mapped the MoCoD locus to be within an 8-cM region extending between, but excluding, markers D6S1641 and D6S1672. The disease-associated haplotype in the patients from kindred 1 was compared with the affected chromosome in the patients from kindred 2. This comparison showed one segment of shared haplotype (D6S1616–D6S1575) and two segments in which the haplotypes differed (D6S1576–D6S1607 and D6S426–D6S282).

Discussion

This is the first linkage study of MoCoD syndrome. We present evidence that a MoCoD locus maps to chromosome 6p21.3, in an 8-cM interval between D6S1641 and D6S1672. Given the small number of patients available, this study emphasizes the power of a dense map of microsatellite markers, combined with homozygosity mapping of rare recessive disease-causing genes, when

genetically isolated populations consisting of large consanguineous families are available (Lander and Boistein 1987; Thomas et al. 1995; Neufeld et al. 1997).

Data associating particular alleles of marker loci with disease-causing mutations are sometimes helpful for the refined localization of a disease gene. When shared haplotypes are found in patients from the same inbred population, this may provide evidence for a founder effect. If there is a founder effect within a population, crossover between the mutation causing the phenotype and closely situated markers—a phenomenon known as "linkage disequilibrium"—is often rare. The region of shared haplotypes in patients from kindreds 1 and 2 may represent this type of linkage disequilibrium (Hastbacka et al. 1992; Sirugo et al. 1992). Haplotype analysis of additional MoCoD-affected families reported in the literature may uncover linkage disequilibrium and may narrow the critical interval. Furthermore, haplotype analysis may give us evidence of genetic heterogeneity, as suggested by the existence of complementation groups, observed in MoCoD patients' fibroblasts (Johnson et al. 1989).

Investigation of the molybdenum-cofactor–synthesis pathway is hampered by the low abundance of the cofactor in the cells and by its extreme instability in isolated form. Genes involved in its synthesis are studied best in eubacteria—namely, *Escherichia coli* K12, for which >12 different corresponding genes have been identified and sequenced (Reiss et al. 1987; Nohno et al. 1988; Johnson et al. 1991*a;* Rivers et al. 1993; Rech et al. 1995). Three genes have been identified in higher plants (Hoff et al. 1995; Stallmeyer et al. 1995): one of them is homologous to the *Drosophila* gene cinnamon and the rat protein gephyrin (Prior et al 1992; Kamdar et al. 1994). The other two plant genes show strong homology to *E. coli* genes involved in the early steps of synthesis, and the complementation studies of human fibroblasts indicate that the majority of MoCoD patients can be expected to carry lesions in similar genes (Johnson et al. 1989). However, no such "early" genes have been identified in mammals. The human homologues of these "early" genes, as well as the human gephyrin gene, are candidate genes for MoCoD.

We have mapped a MoCoD gene to an 8-cM region on chromosome 6p21.3. This region contains several known genes, including the genes for G1/S-specific cyclin D3, peripherin, and progasticsin precursor, and >40 expressed sequence tags (see the NCBI-Science Human Gene Map). However, none of these genes are obvious candidate genes for MoCoD. This suggests that, once refined mapping has been accomplished, identification of the MoCoD gene will require a combination of positional cloning (Collins 1995) and candidate-gene approaches.

MoCoD is an incurable disorder. The cofactor itself is exceedingly unstable. Thus, direct cofactor replacement is not feasible. Various therapeutic trials including oral intake of molybdenum salts and a low-cysteine diet combined with oral sulfate supplementation do not result in any benefit (Munnich et al 1983; Endres et al 1988; Johnson and Wadman 1995). To date, the incidence of this genetic disorder probably is underestimated, because routine metabolic testing may fail to reveal the biochemical abnormalities. An enhanced awareness of this disease and application of the appropriate tests could lead to an increase in the rate of detection of patients, which would reflect MoCoD's true incidence. An excessive occurrence of this fatal disorder was observed among segments of the Arab population in northern Israel. The high incidence probably is due to the high rate of consanguinity (up to 50%) in this population (Jaber et al. 1991) and warrants the urgent development of tools for the detection of carriers, in order to provide genetic counseling. The immediate benefit of our mapping effort is the ability to perform prenatal diagnosis by use of microsatellite markers. This study also provides a tool for carrier detection of this incurable disorder.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

NCBI-Science human gene map, http://www.ncbi.nlm.nih.gov/Science96/ResTools.html

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for MoCoD type A [252150] and type B [252160])

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