Proc. Natl. Acad. Sci. USA
Vol. 77, No. 11, pp. 6715–6719, November 1980
Genetics

Maternal inheritance of human mitochondrial DNA

(genetic polymorphism/restriction endonuclease cleavage map/blood platelets)

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Communicated by L. L. Cavalli-Sforza, July 28, 1980

ABSTRACT **Human mitochondrial DNA was obtained from peripheral blood platelets donated by the members of several independent families. The samples were screened for nucleotide sequence polymorphisms between individuals within these families. In each family in which we were able to detect a distinctly different restriction endonuclease cleavage pattern between the parents, the progeny exhibited the maternal cleavage pattern. Informative polymorphisms were detected for *Hae* II (PuGCGCPy) in a three-generation family composed of 33 members, for *Hin*cII (GTPyPuAC) in a two-generation family composed of four members, and for *Hae* III (GGCC) in a two-generation family composed of four members. The *Hae* II polymorphism was analyzed through all three generations in both the maternal and paternal lines. The results of this study demonstrate that human mitochondrial DNA is maternally inherited. The techniques described for using peripheral blood platelets as a source of human mitochondrial DNA represent a convenient way to obtain data on mitochondrial DNA variation in both individuals and populations.**

Mammalian mitochondrial DNA (mtDNA) is a covalently closed circular molecule that is ≈5 μm long, contains 16.5 kilobase (kb) pairs, and has a molecular mass of 10^7 daltons. Single mammalian cells have been found to contain 1000–10,000 mtDNA molecules (1). The organization of the genes for 12S and 16S rRNA, the origin of replication, and the D loop appear to have been conserved in mammalian mtDNAs (2–6). However, molecular hybridization studies have found considerable sequence divergence among mammalian mtDNAs (7–11), and a recent analysis of changes in restriction endonuclease sites in primate mtDNAs has suggested that mtDNA sequences are changing 5–10 times faster than single-copy nuclear DNA sequences (12). Molecular analysis of nonprimate mammalian mtDNAs also suggests that mtDNAs are changing more rapidly than single-copy nuclear sequence (9).

Maternal inheritance of mtDNA has also been observed in horse–donkey hybrids (13), in the rat *Rattus norvegicus* (14–16), and in the white-footed mouse *Peromyscus polionotus* (17). Maternal inheritance of *Xenopus* and *Drosophilia* mtDNA has also been observed (18, 19). We have taken advantage of the existence of restriction endonuclease mtDNA site polymorphisms within the human population (20–22) and of peripheral blood platelets as a source of human mtDNA (23) to test the hypothesis that human mtDNA is maternally inherited.

MATERIALS AND METHODS

mtDNA Preparation. mtDNA from cultured cells [HT1080 (24) or HeLa S3 (25)] was prepared by using CsCl/ethidium bromide gradients from isolated mitochondria (1, 10). Platelets were isolated from peripheral blood collected from donors after informed consent had been obtained. Thirty milliliters of blood was collected in acid citrate dextrose (ACD in Vacutainer tubes, Becton Dickinson, Rutherford, NJ) and refrigerated. The platelet fraction was isolated within 72 hr of the time of collection. Platelet-rich plasma was collected after centrifugation at 180 × *g* for 15 min at 20°C. Platelets were pelleted by centrifugation at 1000 × *g* for 20 min at 20°C. The platelet pellet was processed by the method routinely used for cultured cell mitochondria or by the following procedure: the platelet pellet was resuspended in 2 ml of buffer (10 mM Tris·HCl/1 mM EDTA, pH 8), 30 μl of 25% $NaDodSO_4$ was added, and the suspension was incubated at 37°C for 10 min. Five microliters of carrier DNA (calf thymus or salmon sperm, 1 mg/ml) in 0.015 M NaCl/0.0015 M Na citrate was added, and the sample was extracted with 0.5 ml of redistilled phenol saturated with 50 mM Tris·HCl (pH 7.6). The phenol layer was back-extracted with 0.1 ml Tris·HCl (pH 7.6) and the aqueous fractions were back-extracted with 0.1 ml of saturated phenol. Then, the aqueous fractions were pooled and extracted twice with an equal volume of anhydrous diethyl ether. The ether was evaporated at 60°C for 15–45 min and the samples were treated with 2 μl of RNase A (10 μg/ml; Sigma R 4875) for 15 min at room temperature. Finally, the sample was extracted with phenol and ether as before and adjusted to contain 0.3 M NaOAc, and the DNA was precipitated by the addition of 2 vol of 95% EtOH and incubation at −80°C. The precipitate was collected by centrifugation at 12,000 × *g* for 5 min, dried at reduced pressure, and resuspended in 25–50 μl of 10 mM Tris·HCl/1 mM EDTA (pH 8).

mtDNA Analysis. Restriction endonuclease digestions were performed in a total volume of 13.3 μl by using reaction conditions and buffers recommended by the manufacturers, except that bovine serum albumin was not used. *Hae* III and *Hin*cII were purchased from New England BioLabs, and *Hae* II was purchased from Bethesda Research Laboratories, Rockville, MD. Sucrose was added to a concentration of 10%, and the digested fragments were separated on a horizontal agarose slab gel (10). The fragments were then transferred to diazobenzyloxymethyl (DBM) paper (26) and hybridized with complementary [^{32}P]RNA (c[^{32}P]RNA) prepared from purified human cultured cell mtDNA banded twice in CsCl/ethidium bromide gradients (10). Fragments were visualized by autoradiography. No cross-hybridization between c[^{32}P]RNA and carrier DNA was observed. For many samples, sufficient mtDNA was present to permit visualization of restriction endonuclease fragments by ethidium bromide staining. The following size standards were used for the determination of restriction endonuclease fragment sizes: λ *Hin*dIII (27); φX174 *Hin*cII (28); and pBR322 *Hae* III (29).

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Abbreviations: mtDNA, mitochondrial DNA; kb, kilobase(s).

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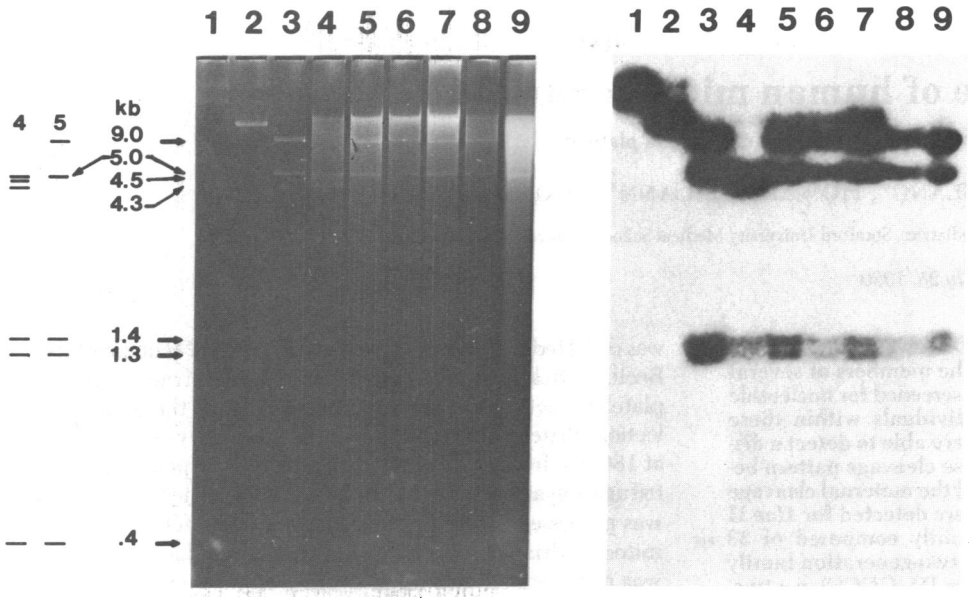

FIG. 1. Maternal inheritance of human mtDNA *Hae* II cleavage pattern. (*Left*) Human mtDNA *Hae* II fragments separated by agarose gel electrophoresis and stained with ethidium bromide. (*Right*) Autoradiograph of *Hae* II fragments from gel after transfer to DBM paper and hybridization with 5×10^6 cpm of human [^{32}P]cRNA (10). Channel 1, HT1080C, uncut; channel 2, HT1080C, *Bam*HI (cuts human mtDNA once); channel 3, HT1080C, *Hae* II; channel 4, father (Fig. 2, I-5); channel 5, mother (I-6); channel 6, son (II-13); channel 7, son (II-14); channel 8, daughter (II-15); and channel 9, daughter (II-16).

RESULTS

Inheritance of *Hae* II mtDNA Polymorphism. The most commonly observed *Hae* II cleavage pattern found in the human platelet mtDNA we have examined (the typical pattern) consists of six fragments ranging in size from 5 to 0.4 kb. In one three-generation family, an atypical fragment pattern was noted that lacked the 4.5-kb and 4.3-kb fragments of the typical pattern and instead contained a 9-kb fragment. The *Hae* II cleavage patterns of a portion of this family are shown in Fig. 1. The female exhibited the atypical pattern (channel 5), her husband exhibited the typical *Hae* II cleavage pattern (channel 4), and their two sons and two daughters all exhibited the atypical maternal *Hae* II cleavage pattern (channels 6–9). We analyzed the *Hae* II mtDNA cleavage pattern exhibited by 33 individuals in this Caucasian family. Three first-generation siblings, two females and a male, expressed the atypical *Hae* II cleavage pattern; only two *Hae* II cleavage patterns were observed in the pedigree of this family (Fig. 2). This situation permitted us to examine the inheritance of the atypical pattern from both the maternal and paternal sides through three generations. The results show that the *Hae* II mtDNA cleavage pattern in the progeny is always identical to the maternal pattern. We infer from this observation that human mtDNA is maternally inherited.

Interestingly, HT1080 cultured human cells exhibited a *Hae* II cleavage pattern similar to the atypical platelet *Hae* II cleavage pattern (Fig. 1), while HeLa S3 cells exhibited a *Hae* II cleavage pattern distinctly different from either the typical or the atypical *Hae* II platelet mtDNA pattern (data not shown). The observation of these different *Hae* II mtDNA polymorphisms suggest that this particular restriction endonuclease may be especially useful for analyzing mtDNA in human populations. A map of the *Hae* II sites (typical and atypical) of human mtDNA is shown in Fig. 3. The atypical *Hae* II cleavage pattern appears to be due to the absence of a *Hae* II site at map position 0.28, which is present in mtDNA from persons exhibiting the typical *Hae* II cleavage pattern.

Inheritance of *Hin*cII mtDNA Polymorphism. The typical human mtDNA cleavage pattern produced by *Hin*cII, described by Brown and Goodman (21), consists of 11 fragments ranging in size from ≈4 kb to <0.2 kb. We observed a difference between the platelet mtDNA *Hin*cII cleavage patterns exhibited by the parents in a two-generation Oriental family. The paternal pattern corresponded to the atypical *Hin*cII mtDNA pattern observed by Brown and Goodman (21), while the maternal pattern corresponded to the typical pattern. Under our conditions of electrophoresis, the smallest *Hin*cII fragment

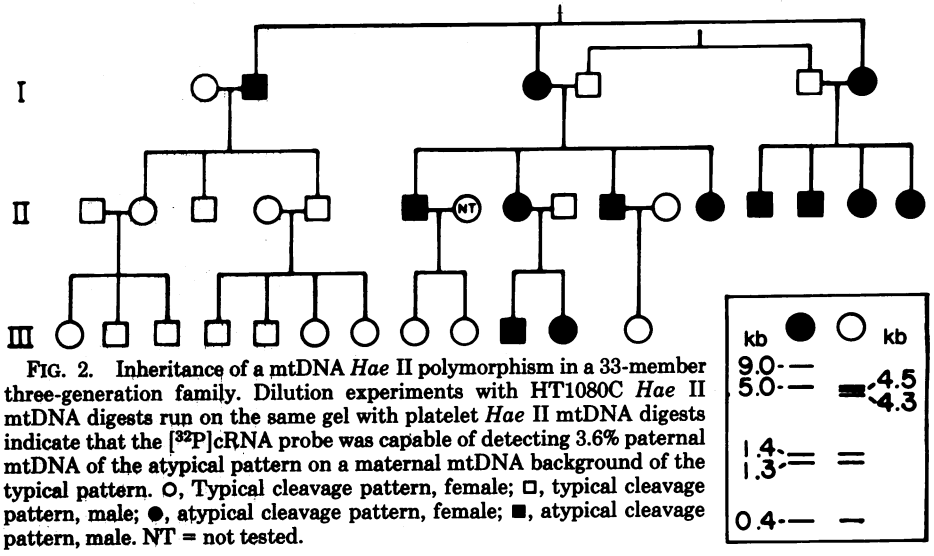

FIG. 2. Inheritance of a mtDNA *Hae* II polymorphism in a 33-member three-generation family. Dilution experiments with HT1080C *Hae* II mtDNA digests run on the same gel with platelet *Hae* II mtDNA digests indicate that the [^{32}P]cRNA probe was capable of detecting 3.6% paternal mtDNA of the atypical pattern on a maternal mtDNA background of the typical pattern. ○, Typical cleavage pattern, female; □, typical cleavage pattern, male; ●, atypical cleavage pattern, female; ■, atypical cleavage pattern, male. NT = not tested.

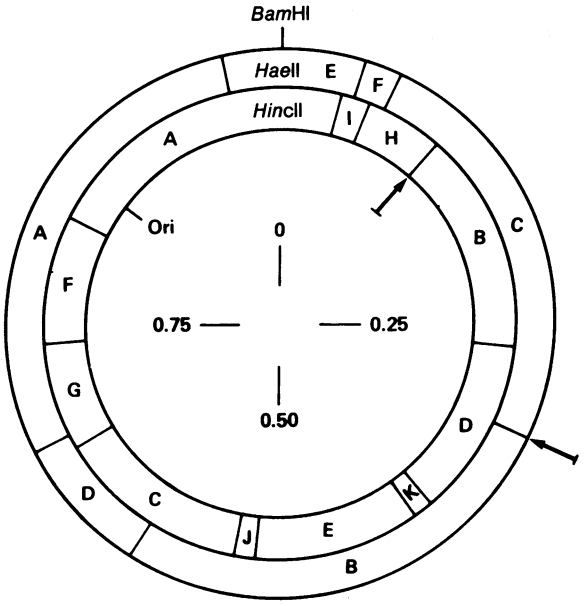

FIG. 3. Restriction endonuclease cleavage map of human mtDNA. (Outer ring) *Hae* II sites: arrow indicates site present in the typical pattern and absent in the atypical pattern. *Hae* II fragments are expressed in kb: A = 5.0; B = 4.5; C = 4.3; D = 1.4; E = 1.3; F = 0.4. (Inner ring) *Hin*cII sites (the arrangement of the fragments is according to ref. 21). Arrow indicates site present in the typical pattern and absent in the atypical pattern. *Hin*cII fragments are expressed in kb: A = 4.1; B = 2.6; C = 2.3; D = 2.0; E = 2.0; F = 1.5; G = 1.3; H = 0.9; I = 0.35; J = 0.26; K = 0.2. The map is oriented with a single *Bam*HI site at 0 and the origin of replication is shown (Ori).

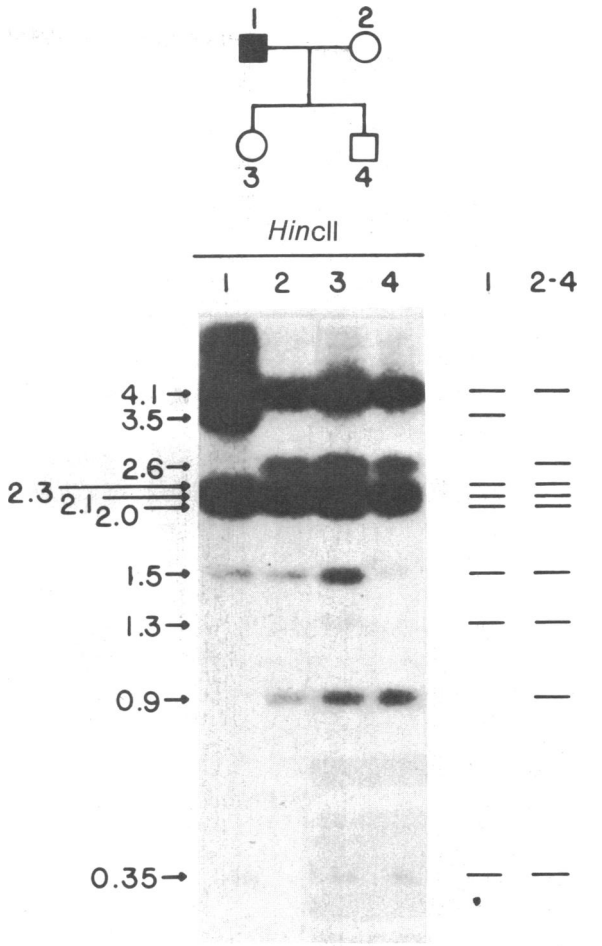

FIG. 4. Maternal inheritance of a mtDNA *Hin*cII polymorphism. Channel 1, father (atypical pattern); channel 2, mother (typical pattern); channel 3, daughter (typical pattern); and channel 4, son (typical pattern). The 1.3-kb fragment present in all four channels was faintly visible in the original autoradiograph, probably due to incomplete transfer or incomplete binding to the DBM paper (this band can be detected by longer exposure, data not shown). ○, Typical cleavage pattern, female; □, typical cleavage pattern, male; ●, atypical cleavage pattern, female; ■, atypical cleavage pattern, male.

(21) was not observed and was probably run off the end of the gel. The difference in these two *Hin*cII fragment patterns appears to be due to the absence of a *Hin*cII site in the typical pattern, which leads to the presence of a 3.5-kb fragment in the atypical pattern rather than the 2.6-kb and 0.9-kb fragments (Fig. 3). Both the son and daughter exhibited the typical *Hin*cII cleavage pattern characteristic of the maternal mtDNA (Fig. 4). Thus, this pedigree, which was studied by using a second restriction endonuclease polymorphism, also demonstrates molecular inheritance of mtDNA.

Inheritance of *Hae* III mtDNA Polymorphism. A third example of maternal inheritance of mtDNA restriction endonuclease pattern was found in a Caucasian family in which the parents differed in their *Hae* III mtDNA cleavage patterns. Both the son and daughter exhibited *Hae* III mtDNA cleavage patterns indistinguishable from the maternal pattern and distinctly different from the paternal pattern (Fig. 5). *Hae* III digestion of human mtDNA gave ≈30 small fragments (20), which have not been mapped and are not well resolved in agarose gels. Therefore, the nature of the *Hae* III site changes that have led to the different paternal and maternal fragment patterns evident in Fig. 5 are unknown.

DISCUSSION

The simplest explanation for our observations on the inheritance of mtDNA restriction endonuclease cleavage patterns in the three families we have studied is that human mtDNA is maternally inherited. In the case of the *Hae* II polymorphism, a dilution experiment indicated that paternal mtDNA would have been detected if present at a level of 4%. This observation excludes a codominant mode of inheritance but does not eliminate the possibility of a small amount of paternal input. X-linked inheritance of the *Hae* II cleavage pattern is excluded. If the atypical pattern were determined by an X-linked dominant gene, then females II-2, III-8, III-9, and III-12 should express the paternal *Hae* II pattern rather than the maternal pattern. If the typical pattern were determined by an X-linked dominant gene, females II-8, II-12, II-15, and II-16 should express the paternal rather than the maternal pattern. An autosomal dominant or recessive mode of inheritance of either *Hae* II cleavage pattern is not completely excluded in the pedigree shown in Fig. 2.

However, this type of inheritance seems unlikely because there is no evidence of segregation for each sibship issuing from every possible mating between a heterozygote and a homozygous recessive individual in generations 1 and 2. Furthermore, in every mating potentially informative for segregation, the *Hae* II cleavage pattern of each offspring is identical to that of the mother, most unusual for autosomal dominant or recessive inheritance. With autosomal genes, there should be no preferential transmission of the maternal or paternal phenotype and hence no association between the phenotype of the progeny and the sex of the parents. The probability of our collecting seven informative families (five for *Hae* II, one for *Hin*cII, and one for *Hae* III) that by chance all show maternal transmission of the mtDNA phenotype would be $(1/2)^7 = 0.0078$. Thus, our

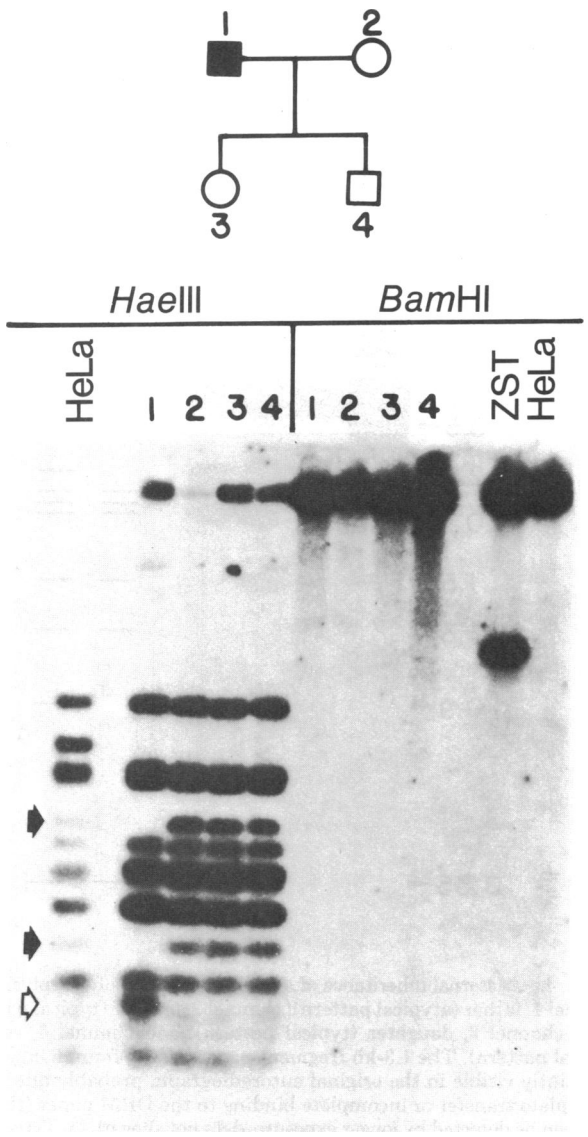

FIG. 5. Maternal inheritance of a mtDNA *Hae* III polymorphism. Closed arrows show bands present in the mother (channel 2) but absent in the father (channel 1) and present in the children (channels 3 and 4). The open arrow shows a band present in the father but absent or greatly reduced in the mother and children. The cultured human cell line HeLa derived from a black woman is seen to have a *Hae* III pattern different from both of the parents. Digestion of the same platelet mtDNA samples with *Bam*HI which, in typical human mtDNAs, cleaves once, indicates that the high-molecular-weight fragments present in all four *Hae* III channels are incompletely digested mtDNA. A *Bam*HI polymorphism has been found to be present in our human fibroblast line ZST (previous *Bam*HI polymorphisms have been described, see ref. 21). ○, Typical cleavage pattern, female; □, typical cleavage pattern, male; ●, atypical cleavage pattern, female; ■, atypical cleavage pattern, male.

results show that there is a strong association between the phenotype of the offspring and that of the mother. Because such an association would not be expected for autosomal genes, it is unlikely that this inheritance pattern is autosomal in nature. In conclusion, the *Hae* II pedigree has permitted exclusion of X-linked inheritance, all three pedigrees are inconsistent with codominant inheritance, and the cumulative results render autosomal modes of inheritance unlikely. Only a maternal mode of inheritance is in complete agreement with the data.

In addition to showing that human mtDNA is maternally inherited, our results show that small quantities of peripheral blood are sufficient to permit use of restriction endonucleases in the study of human mtDNA in populations comparable to previous studies on *Peromyscus* sp and the pocket gopher (17, 30). We normally obtain approximately 1 μg of mtDNA from the platelets collected from 30 ml of blood. This quantity of mtDNA is sufficient for a number of restriction endonuclease digestions when the fragments are detected by using radioactive probes. mtDNA is also readily detected by hybridization in restriction endonuclease digests of whole cell DNA prepared from the total leukocytes from 30 ml of blood (data not shown). Thus, in genetic studies of human populations, mtDNA polymorphisms may either be analyzed in DNA prepared from peripheral blood platelets (which permits using the remaining erythrocytes and leukocytes for testing other biochemical markers of interest) or in DNA prepared from whole leukocytes (which is compatible with simultaneous testing using other nuclear DNA probes). The ability to follow the inheritance of human mtDNA in families by analyzing blood samples for mtDNA restriction endonuclease site polymorphisms also provides an experimental approach for analyzing the genetic basis of inborn aberrations associated with mitochondrial dysfunction, such as familial mitochondrial myopathy (31, 32), which may be due to alterations in genes located on the mtDNA.

We thank Mrs. Minna Moore-Benedict for recruiting participants in these family studies and Mrs. Loverine Taylor for assisting in the construction of the human mtDNA Hae II map. We would also like to acknowledge the technical assistance of Mrs. Betty van West and Mr. Hung Pham. This work was supported by National Institutes of Health Grants GM 28428 and GM 24285, March of Dimes–National Foundation Basil O'Conner Starter Grant 5-219, National Science Foundation Grant PCM 77-24793 to D.C.W., and National Institutes of Health Postdoctoral Fellowship 5T32 CA 09302-03 to R.E.G.

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