MINIREVIEW

Noninsulin-Dependent Diabetes Mellitus as a Mitochondrial Genomic Disease (44321)

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Abstract. **The genetics of diabetes mellitus is very complex. Although the phenotypes are relatively simple *vis-à-vis* an abnormal glucose-insulin relationship, a number of genotypes share this phenotype. This review focuses on mutations in the mitochondrial genome that phenotype as diabetes mellitus. Studies in the human and the rat are described.** [P.S.E.B.M. 1998, Vol 219]

Diabetes mellitus can arise from any one of a number of mutations in either the nuclear or mitochondrial genomes. Thus, this term, diabetes mellitus refers to a group of genetic disorders having in common a disordered glucose-insulin relationship. Secondary complications involving the circulatory system, the central nervous system, and the renal system are common, yet not all people with diabetes mellitus develop all the additional symptoms and problems. In some (but not all), obesity is a concurrent problem and indeed may precede the abnormal glucose tolerance that characterizes the individual with diabetes. Other diseases such as heart disease and renal disease may also develop concurrently or sequentially depending on the specific genetic disorder that caused the diabetic state. This review describes and discusses the diabetic condition that arises as a result of a mutation in the mitochondrial genome. This form of diabetes is transmitted as a maternal trait because mitochondrial (mt) DNA is of maternal origin (1). Very little mtDNA is contributed by the sperm because the mitochondria in the sperm are located in the tail. Only the head of the sperm penetrates the egg at conception. Thus mt mutations are inherited from the mother—not the father.

The mitochondrial genome serves as a site for a number of genetically determined degenerative diseases in addition to diabetes mellitus. Some of these diseases are relatively rare whereas some (i.e., diabetes) "may" be quite common. The may is in quotation marks because the prevalence of these mutations is only now being widely studied. As with any genetic disease, the severity and time course of development is determined by the location of the mutation within a specific gene. A mutation in a part of the gene that is translated into an active portion of the resultant protein would be far more devastating than a mutation in a more distal location. In turn, those mutations that result in devastating lethal diseases are rare and usually the result of a spontaneous mutation in that individual. Such a disease markedly shortens the life span of the individual who, in turn, would not likely contribute this gene mutation to the population's gene pool.

In contrast to diseases caused by mutations in nuclear DNA, mutations in mtDNA might not be fully expressed. This is because there are many mitochondria in each cell. The liver, for example, has between 500–2500 mitochondria per cell with the average value of about 1300. Each mitochondrion has about eight copies of the genome. Cells differ in the number of copies; thus, the range is broad (1000–10,000 copies/cell). This is in contrast to nuclear DNA of which there are only two copies per cell. If some of the mtDNA copies have a normal base sequence and others have a mutated sequence, the cell is heteroplasmic. Homoplasmic cells are those containing mtDNA with identical

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sequences. If the mutation is a point mutation, that is, a substitution of a base that in turn results in an amino acid substitution in the translation product, this mutated DNA will coexist with normal DNA in the same cell. Depending on where the base pair substitution occurs with respect to the active site of the translation product, the effect of the mutation could be minimal, modest, or profound with respect to the function of that particular mitochondrion. As mentioned, there are many mitochondria in any given cell so if a small percentage is aberrant, the existence of the mutation might not be known. However, should a large percentage be aberrant, there could be measurable consequences. Whereas a number of nuclear mutations have been identified that associate with NIDDM, there also is a growing body of literature that shows that mutations in the mitochondrial genome also associate with this disorder.

The Mitochondrial Genome

In contrast to nuclear DNA (nDNA), which exists as a linear molecule, mtDNA is a closed circular molecule. The size of mtDNA ranges from approximately 16 kb in animals to more than 100 kb in plants (2). Mitochondrial DNA in animals is nearly identical in size and has the same organization and content of genes, which encode homologous products (3–12). In the rat, the size of the mitochondrial chromosome is 16,298 bp; in the human it is 16,569 bp (12). The mitochondrial genome has been sequenced and mapped in many organisms including the human and the rat (4–12). Figure 1 illustrates the locations of each of the genes on the mitochondrial chromosome. This DNA encodes 22 tRNAs, 2 ribosomal RNAs, and 13 structural genes that are all components of oxidative phosphorylation (OXPHOS) (2, 5, 7, 8, 10–14). The structural genes include 7 of the 39 units of Complex I (ND1, 2, 3, 4, 4L, 5, and 6), 1 of the 10 units of Complex III (Cyt b), 3 of the 13 units of Complex IV, Cytochrome C Oxidase (CO I-IV) of the respiratory chain, and 2 units of the F_O portion of Complex V, the F_1F_OATPase. Approximately 6% of the bases in the mitochondrial genome are noncoding. Ninety-seven percent of this noncoding DNA is located in the two controller regions,

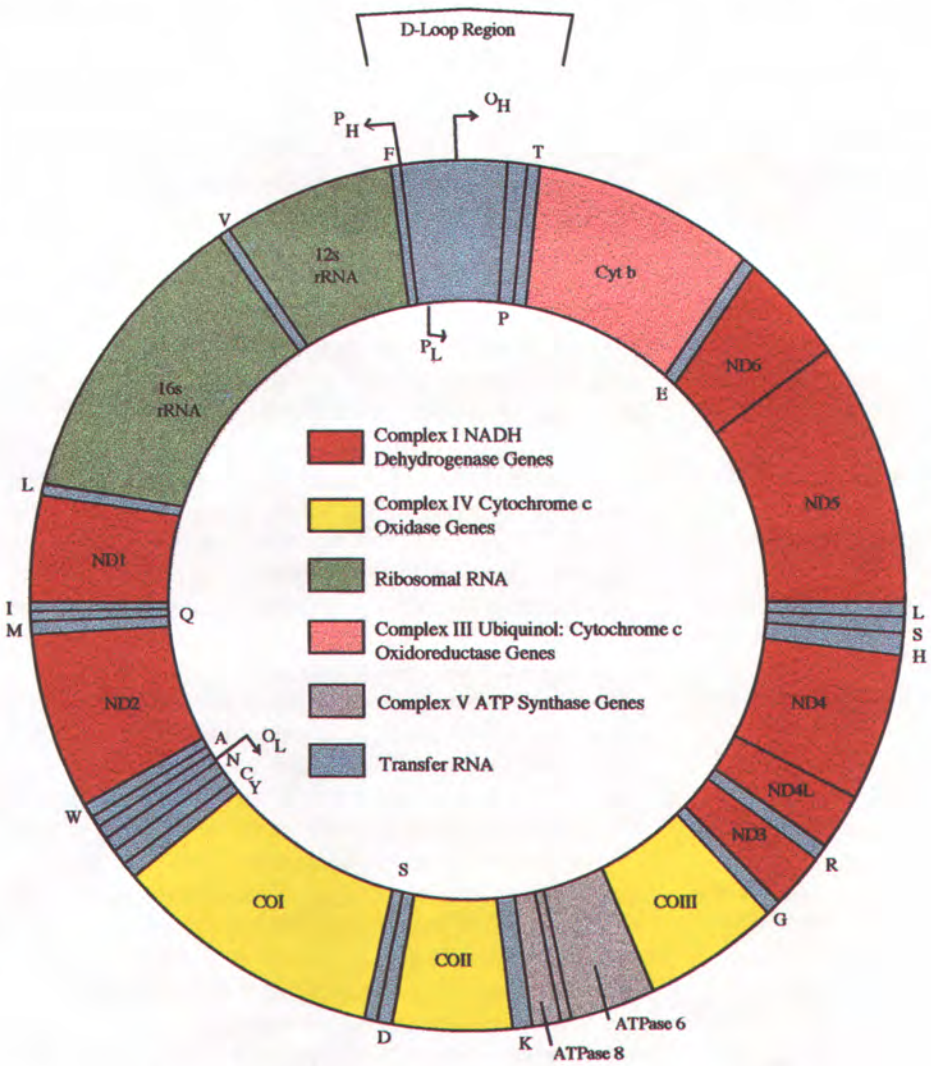

Figure 1. Transfer RNAs are indicated by single-letter amino acid code with these encoded by the light strand on the inside and those encoded by the heavy strand on the outside of the circle. OL, origin of light strand; OH, origin of heavy strand; PH, heavy strand promotor; PL, light strand promotor.

the 30-bp origin of replication for the light strand (O_L), and the 898-bp unit called the displacement loop or the D-loop.

The D-loop region is sandwiched between the genes for $tRNA^{Phe}$, which is downstream, and $tRNA^{Pro}$, which is upstream (14). This control unit contains the origin of replication for the heavy strand (O_H) (4), the origins of both heavy and light strand transcription (5), major dedicated promoters for both heavy and light strand transcription (6, 13), two transcription factor binding sites, three conserved sequence blocks associated with the initiation of replication, and the D-loop strand termination associated sequences (15, 16). The light strand promoter region serves two purposes; it is not only the major promoter for light strand transcription, but also the site of priming for leading strand mtDNA replication.

The entire length of the genome contains genes situated on both strands. The heavy strand is the main coding strand, and codes for 2 rRNA species, 14 tRNA species and 12 structural genes. The light strand codes for 8 tRNA species and 1 structural gene for a subunit of NADH dehydrogenase (ND6) (14). The genome sequence shows extreme economy in that the genes have few noncoding bases between them (4). In fact, the largest space between two genes in mtDNA (excluding the controller regions) is the five-base pair sequence between the genes for $tRNA^{Glu}$ and the cytochrome *b* gene. There is gene overlap as well. In six cases, there are genes that share coding nucleotides. In half of the cases, the shared nucleotides are not really shared; they are on the heavy and light strands such that the shared bases are on the 3′ ends of two genes. In the other three cases, the shared genes are actually shifts in the reading frame to allow the same nucleotides to code for two different proteins. The genes that share nucleotides include the ATPase 6 and 8 genes that share 52 base pairs, ND4 and ND4L that share 7, the $tRNA^{Ser}_{Agn}$ and $tRNA^{Leu}$ that share 1, ND5 and ND6* that share 31, the $tRNA^{Ser}_{UNC}$ and the cytochrome oxidase I gene that share 4, and lastly, the $tRNA^{Ile}$ and $tRNA^{Gln}$ that share 3. Interestingly, only 56 base pairs are all that separate the different genes on the mt chromosome; 98 base pairs are shared by neighboring genes.

The initiator codon of each reading frame, which can be either AUG, AUA, AUU, or AUC, follows immediately or only a few bases after the 3′ termination sequence of its upstream neighbor. Most reading frames lack termination codons and only code a T or a TA after the last sense codon. Completion of the termination codon occurs at the time of RNA processing by polyadenylation (17). Introns, common in nDNA, are not a feature of mtDNA. The tRNA genes are used as intervening sequences and come into play during RNA processing. Transfer RNA removal from primary transcripts results in the production of both the tRNA species themselves as well as mature mRNAs (18).

The mt genome of most mammals has a GC content between 36% and 44%. In the rat, the infrequent occurrence of guanine in the third codon position reflects the generally low amount of this base in the genome. Some G ending codons (i.e., TAG, AGG) are not used at all in this species. ATG is fairly abundant because of its function as an initiator codon. TAA is used as the stop codon in the mtDNA. One of the peculiar features of the mtDNA is the use of TGA as the codon for tryptophan. Considering the number of tRNAs present in this organelle, wobble rules have been determined. In the sets of four synonymous codons, an unmodified U exists in the first position of the anticodon and "wobble-pairs" with all four bases in the third position of the codon (19). Uridine is the only base that can form a "stable" pair with all four nucleotides in the third position of the codon in either a two out of three or a U:N wobble (20). In two-codon sets, post-transcriptional modifications restrict the pairing of U to A and G (21). For each of the eight "genetic code boxes" containing four synonymous codons, there is a single specific tRNA gene with a T in the first position of the anticodon (22). In the case of tRNA genes corresponding to the sets of two synonymous codons ending with a purine, a T is also found at the first position of the anticodon, and it is thought that the corresponding U in the tRNAs is modified to restrict the codon recognition to two codons (23).

Nuclear encoded enzymes and proteins play an important role in the transcription of mtDNA, the processing of mtRNAs, and the translation of mitochondrial messages. These additional nuclear encoded enzymes are needed for the synthesis of the entire complement of mitochondrially encoded proteins. Other nuclear genes also have functions that relate to the expression of individual mitochondrial gene products such as cytochrome *b* and the subunits 1 and 2 of cytochrome oxidase (24). The expression of these genes depends on nuclear genes that code for proteins involved in 5′-end processing (25), intron excision of the pre-mRNA (25, 26), and translation of the mature message (27).

In addition to the above, the development of functional mitochondria requires structural and regulatory genes called PET genes located in the nucleus (28). The expression of these genes is required for the biogenesis of respiration-competent mitochondria. These genes code for products that have a direct function in mitochondrial respiration and ATP synthesis, yet they affect mitochondrial oxidative metabolism indirectly.

The fact that genetic information is distributed among two spatially separate compartments implies the existence of some mechanism for ensuring a coordinate expression of the proteins and/or RNAs encoded in the two genomes. Growth and division of eukaryotic cells are accompanied by a concomitant increase in mitochondrial mass. This is accomplished not only by the *de novo* formation of the organelle, but also by the addition of lipid and newly synthesized proteins to preexisting mitochondria (28). The latter condition depends on the balanced rates of synthesis of both the nuclear and mitochondrially encoded constituents of this organelle. In some single-celled organisms such as yeast

(29), mtDNA may be absent, yet the organism is able to function somewhat. Some compensatory reactions serve as respiratory components. In complex animals, compensation can occur in respiration-deficient cells; however this compensation is quite limited.

Lastly, the transcription of mtDNA is controlled not only by nuclear-encoded transcription factors but also by mitochondrial-specific factors that seem to target mtDNA (6, 30, 31). Thyroid hormone appears to act in this way, but the mechanism of its action has not been clearly described. Other mtDNA binding molecules have been suggested, but the details of such regulatory effects are lacking.

Mutations in mtDNA

Just as mutations in the nuclear DNA can result in discernible disease, so too can mutations[3] in the mtDNA. A number of reviews in the literature have described the diseases that associate with these mutations. Some of these are cited in this review (e.g., 2, 9, 32–36 and others). Both heteroplasmic and homoplasmic mutations have been reported, and these mutations include both point mutations and deletion mutations. Large and small deletion mutations have been reported (7, 32–35). Deletion mutations can occur due to slipped mispairing between repeated sequencess during DNA replication or by erroneous RNA splicing (30). Domains containing tandemly repeated DNA sequences are often highly polymorphic in length due to the propensity of repeat units to undergo addition or deletion events (7, 12, 32, 33, 34, 36–42). Slipped mispairing between adjacent or nearby repeat sequences during replication is one of several proposed mechanisms for mtDNA deletion mutation. Slipped mispairing between distant repeats can also occur and may be responsible for larger-scale deletions associated with a variety of neuromuscular diseases in humans (12, 32, 33, 35). Deletions can also occur as a result of free radical attack on the genome (37). Age-dependent deletion has been reported in human liver (36–39), and these deletions were found to parallel an age-related decline in respiratory function (37).

Madson *et al.* (35) have investigated the mechanism of slipped mispairing. They analyzed a repeat domain present in porcine mtDNA. This domain was located at the 5′ end of the D-loop between conserved sequence blocks 1 and 2. This sequence consisted of 14–29 copies of a 10-bp, self-complimentary, tandemly repeated sequence, CGTGCGTACA. Upon passage into *E. coli,* a recombinant plasmid containing this domain displayed a unique polymorphic pattern that was different from that seen in the pig. Using either single- or double-stranded templates containing the repeat domain, these investigators showed that slippage replication could account for the observed mammalian deletion mutation. Because certain genomes may have more areas of direct repeats and because deletions are more likely to occur in or near these areas, these genomes might be more vulnerable to this type of mutation (7, 8, 15, 35, 40–43).

Just as cells can be heteroplasmic with respect to normal mtDNA and DNA with a point mutation, so too can cells be heteroplasmic with respect to mtDNA that has a deletion mutation. However, in the instance of the heteroplasmic cell with the deletion mutation, there is the tendency to drift towards deletion mutation homoplasmy. The reason this drift occurs is because mitochondria reproduce themselves at a rate that is 5 to 10 times faster than the rate of cell replication (15), and shorter strands of DNA are replicated at a faster rate than strands of normal length (16). Thus, a deletion mutation might become evident faster than a point mutation—all other factors being equal. In this scenario, age is a critical determinant of the percentage of mtDNA with a deletion mutation. As the animal or human ages, there is a drift toward deletion mutation homoplasmy. Several investigators have reported that age, as well as dietary fat as a source of free radicals, are critical factors in the accumulation of deletion mutation (36–40, 43–45).

Point mutation in the codes for the 13 structural genes as well as in the codes for the tRNAs have been reported as well as mutations in the controller regions (46–51). Some of these associate with diabetes mellitus. Controller region sequence mutation has been reported to occur at rates faster than mutation rates elsewhere in the genome. Rates of base-pair substitution in this region vary among the different sites of this region, and these sites are distributed along the region rather than being clustered (50). Polymorphic variation occurs as well with few or no effects on the activities of the gene products (50, 51).

Mitochondrial DNA Mutations that Associate with Diabetes Mellitus in Humans

NIDDM has a strong genetic component as suggested by a concordance rate that approaches 100% with age in monozygotic twins (52, 53). Whereas this high concordance rate indicates that the disease has a strong genetic link, the mode of inheritance is highly variable. NIDDM has a multifactorial etiology in which environmental factors, such as diet, are important modifiers. NIDDM can be thought of as a disease that results from a nutrient-gene interaction. For some forms of NIDDM, the genetic clues have been discovered. These forms are usually of the early-onset type and are monogenic as well as uncommon. Listed in Table I are some of the nDNA mutations that have been identified as being associated with NIDDM. These mutations, although numerous, account for less than 5% of the individuals with

[3] Mitochondrial diseases due to mutations in mtDNA are abbreviated as follows: NIDDM, noninsulin-dependent diabetes mellitus (Type II); MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms; LHON, Leber hereditary optic neuropathy; MERRF, myotonic epilepsy and ragged red fiber disease; IDDM, insulin-dependent diabetes mellitus; NARP, neurogenic muscle weakness, atoxia, retinitis pigmentosa. Complete descriptions of these diseases and their associated mutations can be found in references 2, 9, 33, 36–40, 46–49, 68–74, 87–101 as well as in many other reviews and reports not cited due to space limitations.

[4] Other abbreviations used: bp, base pair; mt, mitochondria; mtDNA, mitochondrial DNA; ND1–6, NADH oxidoreductases that are part of respiratory chain complex I, the NADH ubiquinone oxidoreductase; COX, cytochrome oxidase; OXPHOS, oxidative phosphorylation.

Table I. Nuclear DNA Mutations Associated with NIDDM

Gene	Chromosome location	Comment
Glucokinase (MODY 2)	7	Uncommon; heterogeneous.
Insulin cleavage enzyme	?	Results in excessive amounts of proinsulin in the blood.
MODY 3	12q	Encodes transcription factor 1α.
MODY 1	20	Encodes transcription factor 4α.
Glycogen synthase	19q 13.3	Two forms (liver & muscle); Highly polymorphic.
FAD-glycerol 3 phosphate dehyrogenase	3	Associates with NIDDM in the GK rat.
Glucagon receptor	?	Associates with NIDDM in French and Sardinian subjects but not Japanese subjects.
CCKBR	11p 15.4	Associates with NIDDM in French subjects.
IRS 1	?	Plays a role in downstream insulin signaling. When mutated, the signal pathway is aberrant.

NIDDM. This group includes individuals with maturity onset diabetes of the young (MODY), which is itself a heterogeneous syndrome (54–58). Genes affected in MODY patients include glucokinase (hexokinase IV) (MODY2), as well as the transcription factors, HNF-1α (MODY3) and HNF-4α (MODY1). Heterogeneity is seen in these subgroups. For example, more than 44 different mutations in MODY-2 patients have been reported (54–56). Mutation in the gene for insulin (59), the genes for insulin processing enzymes (60), and the genes for the insulin receptor (61) have also been detected and associated with the early onset forms of the disease.

The maternal inheritance pattern of diabetes has led researchers to look for genetic defects in the mitochondrial genome. As mentioned earlier, mtDNA is maternally inherited because the mitochondria contributed to the embryo by the sperm is much less than that present in the egg at the time of fertilization. The ratio is around 1:1000. Thus, mtDNA mutation(s) are transmitted to the progeny *via* maternal lineage. Mutations in the mitochondrial genome should have an impact on cellular energy production as genes encoded by the mitochondrial genome are components of oxidative phosphorylation (OXPHOS) or are their supporting tRNAs or rRNAs. Inhibition of cellular energy production has been shown to reduce or abolish both insulin secretion and action (62–65) and of course, this is central to the diabetic condition. In addition, losses in OXPHOS efficiency mean that there are losses in the control of glucose homeostasis as evidenced by elevations in the tissue and blood lactate levels as well as impaired glucose tolerance.

To date, 42 different mitochondrial DNA mutations (point mutations, deletions, and duplications) have been found to associate with the NIDDM phenotype (Table II). Many are associated with other mitochondrial syndromes and involve disturbances in CNS function as well as disturbed muscle and renal function. In these instances, the NIDDM symptoms are less important than those associated with the CNS. Patients with NIDDM associated with mtDNA mutations generally are not obese (66). They exhibit hyperglycemia that is due to a significantly reduced insulin secretory capacity that progresses with age (67–74).

Table II. Point Mutations in mtDNA exclusive of the ATPase genes that Manifest Primarily as Diabetes Mellitus

Position	Mutation[a]	Gene
3243[a]	A→G	$tRNA^{Leu}$
3252	A→G	$tRNA^{Leu}$
3256	C→T	$tRNA^{Leu}$
3271	T→C	$tRNA^{Leu}$
3290	T→C	$tRNA^{Leu}$
3291	T→C	$tRNA^{Leu}$
3316	G→A	ND1
3348	A→G	ND1
3394	T→C	ND1
3396	T→C	ND1
3423	G→T	ND1
3434	A→G	ND1
3438	G→A	ND1
3447	A→G	ND1
3480	A→G	ND1
3483	G→A	ND1
4216	T→C	ND1
4917	A→G	ND2
5780	G→A	$tRNA^{Cys}$
7476	C→T	$tRNA^{Ser}$
8245	A→G	COX II
8251	G→A	COX II
8344	A→G	$tRNA^{Lys}$
10398	C→T	ND3
11778	T→C	ND4
12308	A→G	$tRNA^{Leu(cun)}$
14709	T→C	$tRNA^{Glu}$
15904	C→T	$tRNA^{thr}$
15924	A→G	$tRNA^{thr}$
15927	G→A	$tRNA^{thr}$
15928	G→A	$tRNA^{thr}$
16069	C→T	D-loop
16093	T→C	D-loop
16126	C→T	D-loop

[a] When the mutation is heteroplasmic, NIDDM rather than a more serious disease develops if the percentage mutation is less than 50%.

This hyperglycemia is not due to decreased insulin sensitivity (74), aberrant insulin receptors, or aberrant mobile glucose transporters (74–76). These patients develop a fatty

liver, which is not an uncommon feature of diabetes mellitus (73). β-Cell loss is seen (77) as well as defects in glucose-induced signaling of insulin release (47, 77, 78).

The mitochondrial $tRNA^{Leu(UUR)}$ gene is an etiological hot spot for mtDNA mutations. So far, eleven disease-related mutations in this gene have been described, with six associated with NIDDM. Mutations in this particular gene comprise 60% of all of the mitochondrial tRNA gene mutations and collectively account for about 10% of all people with NIDDM (79). An A/G transition at bp 3252 was found to be associated with mitochondrial encephalomyopathy, pigmentary retinopathy, dementia, hypothyroidism, and NIDDM (80–83). Another A/G point mutation at bp 3243 has been associated with maternally inherited myopathy and cardiopathy (33) and with MELAS (79). A C/T exchange at 3256 in a patient with a MERRF-like syndrome has also been reported (79). It is interesting to note that the 10 different mutations in the same gene can have different phenotypes. The differences in the phenotypes are due to the degree of heteroplasmy, the position of the mutation, and the environmental influences such as diet. The mutations in the tRNA are located in a region of the DNA responsible for transcription termination of the rRNA genes and as such, affect not only the amino acid sequences of the structural genes but also the termination by one of several mechanisms as proposed by Moraes *et al.* (79). These include interference with 16S rRNA synthesis, alteration in the mRNA:rRNA ratios, or overproduction of the termination factor interfering with light-strand transcription. Any or all of these mechanisms could be operative and result in disrupted mitochondrial OXPHOS function due to aberrant mt gene products.

In 1989 a particular pedigree was observed in which all nine children of a mother with NIDDM also exhibited the disease (81). The children had accompanying hearing loss, yet the severity of the NIDDM varied. When members of the third generation were studied, it was discovered that the diabetes and deafness were present in the children of affected mothers not affected fathers. This maternal inheritance pattern is the hallmark of syndromes associated with mtDNA mutations. This mutation was heteroplasmic with an A to G substitution at position 3243 in the $tRNA^{Leu(UUR)}$ gene (49, 82, 83). Subsequently, this particular base substitution has been found in a number of diabetic populations and accounts for ~1.4% of all of the diabetes cases where known genetic causes have been identified.

NIDDM has been reported as a secondary characteristic in many of the mitochondrial diseases. Patients with chronic progressive external ophthalmoplegia (CEOP), and the complete Kearns-Sayre syndrome carrying large mtDNA deletions and duplications (84–88) have been reported along with NIDDM. Patients with Pearsons disease that involves the bone marrow as well as the pancreas (89) and patients with renal tubular dysfunction (90, 91) also exhibit diabetic symptoms. Glucose intolerance or frank diabetes mellitus occurs in some subjects with mitochondrial myopathy (75, 92, 93). Of particular interest is the syndrome, MELAS, which shares the point mutation at position 3243 in mtDNA with NIDDM. Persons with NIDDM as a result of the 3243 mutation may have no clinical neurological defects (94, 95). The severity of the symptoms are directly related to the percentage of mtDNA that is aberrant. Those with MELAS have a large percentage of their tRNA with the mutation whereas those with NIDDM (only) have a much smaller percentage of mutated tRNA. MELAS, LHON, and MERF also develop as a result of mutations with the structural genes comprising the respiratory chain. Mutations in the any of these genes result in nonfunctional or partially functional respiratory activity and, as a result, ATP production is impaired. Because the CNS depends so heavily on ATP production, large-scale reductions in synthetic capacity will affect this system before any other system. Other organs and tissues that have alternative and compensatory mechanisms available for maintaining, at least in part, a "near normal" phosphorylation state will be less seriously compromised. Yet even these mechanisms will fail as the person ages, and when this failure occurs, clinical symptoms of abnormal glucose homeostasis will be observed.

Actually, any mutation of the mitochondrial DNA should affect the synthesis of ATP and, therefore, the supply of cellular energy. Of interest to the current work on mtDNA mutation and NIDDM is the discovery that mutations in the mtATPase 6 and 8 genes associate with NIDDM. To date, five mutations that associate with NIDDM (Table III) have been reported in the genes for ATPase 6 and 8 (89, 95–101). These two subunits along with the 10 nuclear encoded subunits assemble to form complex V of oxidative phosphorylation, the E_1F_OATPase. This complex captures the energy generated by the respiratory chain as it joins two hydrogens to an oxygen to form water. Some of the energy is released as heat whereas the remainder is used to form ATP. ATP synthesis is crucial for

Table III. ATPase 6 Mutations in Humans and Rats that Associate with NIDDM

Position	Mutation	Species	Comments
8993	T→G	Human	Heteroplasmic: Accounts for 0.4%–10% of NIDDM populations surveyed.
8993	T→C	Human	Not as common as the T→G mutation; heteroplasmic.
8860	A→G	Human	Similar in position to the rat mutation at 8204.
8894	G→A	Human	Cardiomyopathy is a major feature of this disorder.
8204	G→A	Rat	Homoplasmic; found in BHE/cdb and CFI:CD(SD)BR rats.
8289	C→T	Rat	Homoplasmic; found in the BHE/cdb rat.

the synthesis of protein, especially insulin (102–104), and the intracellular concentrations of ATP are very important for insulin release (46–48, 62, 64, 103). Of course, ATP synthesis is an important component in the regulation of phosphorylation state that in itself has control properties with respect to intermediary metabolism. Shown in Table III are the ATPase mutations that have been found in humans and rats. Note that the human and rat ATPase 6 genes are of different lengths. The human ATPase 6 gene has 636 bp, and the rat has 679 bp. Thus, direct species comparison is not possible. Nonetheless, if one were to compare the approximate locations and effects of these mutations, some benefit could be derived. For example, the mutation at bp 8860 in the human is roughly equivalent to the mutation at bp 8204 in the rat (100, 104). In each species the mutation has occurred at about the same place in the gene and, while not identical with respect to base sequence, each has the same net effect of changing the polarity of the amino acids coded by the base substitution and in each, this amino acid substitution occurs in the proton channel that is so important in the capture of the energy of the respiratory chain and its transmittance to the high energy bond of ATP. Because of this similarity between the two species, the BHE/Cdb rat has become an important tool for the study of mt diabetes. Another rat strain, a derivation of the Sprague-Dawley strain (CFI:CD(SD)BR) maintained by WIGA in Sulzfeld Germany has the same base substitution at 8204 as the BHE/Cdb rat (105). It is also a hyperglycemic, hyperlipemic rat, but its metabolic characteristics have not been throughly studied.

The BHE/Cdb Rat: A rat with mtDNA Mutation in the ATP 6 Gene

The BHE-Cdb rat mimics the human with NIDDM (106–108). It develops moderate hyperglycemia and impaired glucose tolerance as it ages as well as a number of diabetic complications. Renal disease is the primary cause of death. BHE/Cdb rats have an average life span of 600–700 days if fed a stock diet (Purina Chow). The usual life span of Wistar or Sprague-Dawley rats provided the same diet is 1200–1400 days. If BHE/Cdb rats are fed purified diets, they have an earlier onset of glucose intolerance, and their life span is shortened (109–111). If the animals are fed a purified diet with a composition similar to that consumed by humans, the impaired glucose tolerance appears at 100 days of age, and in this setting, 100% of the animals are intolerant (111).

Prior to the development of glucose intolerance, various hepatic abnormalities in metabolic control were observed. Among these were: a two-fold increase in hepatic *de novo* fatty acid and cholesterol synthesis (112) and lipolysis (113), a 40% increase in gluconeogenesis (114), and a 20% reduction in ATP synthesis efficiency (115). In an experiment by Fowler and Berdanier (116), Sprague-Dawley rats were fed a sucrose-rich diet that contained 0.02% 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a mild OXPHOS uncoupling agent. After 8 weeks, the rate of hepatic fatty acid synthesis (tritium incorporation into fatty acids) was increased two-fold in the rats treated with DCMU. They also had body composition changes in that those rats fed the DCMU had slightly more body lipid and slightly less body protein. This experiment suggested that some of the metabolic characteristics previously found in the BHE/Cdb rats could be due to a decrease in the efficiency of coupling respiration to ATP synthesis.

Feeding BHE/Cdb rats purified diets containing sucrose as the carbohydrate source and coconut oil as the fat source resulted in a further increase in fatty acid synthesis, an increase in gluconeogenesis, and a further disruption in the coupling of mitochondrial respiration (117–121). Thyroxin, which has been shown to increase the synthesis of a variety of proteins in OXPHOS, was administered to "rescue" respiration (122–124). An increase in shuttle activity and Mg-ATPase activity were observed, but no increase in coupling efficiency was recorded. In fact, a deterioration in coupling efficiency was observed (118, 119). With some substrates, the mitochondria respired normally, but the energy generated by the respiratory chain was not fully captured in the high energy bond of ATP, implying an error in the ATPase. The observation that the Mg-ATPase was increased with thyroxin treatment showed that the F_1 portion of the ATP synthase was functioning normally and that the F_1F_OATPase complex had been induced by the hormone treatment. This ATPase is the F_1 working in the opposite direction. As a result of these observations, it appeared that the uncoupling occurred because the F_O, although increased in amount, not fully functional. This then led us to examine the portion of the mtDNA that codes for F_OATP synthase subunits 6 and 8.

The mitochondrial (mt) genes for the subunits ATP synthase 6 and 8 were sequenced from BHE/Cdb and Sprague-Dawley rats at 50 and 300 days of age (100). Four single base differences were detected when comparing BHE/Cdb to Sprague-Dawley rats. The base sequences in the Sprague-Dawley rats were identical to those of Gadaleta *et al.* (14). Three of the mutations (8083, 8140, 8251) are probably silent as the base substitution would have no effect on the amino acid sequence of the resultant protein. The tRNA for the amino acids concerned would recognize both the normal and mutant codons, and the appropriate amino acids would be incorporated into the protein being synthesized. The mutation at bp 8204, however, is not silent. At this position the BHE/Cdb cell codes for adenine whereas the Sprague-Dawley codes for guanine. The Sprague-Dawley codon is recognized by the tRNA for aspartic acid whereas the BHE/Cdb codon is recognized by the tRNA for asparagine. This means that there is an amino acid substitution in the F_OATPase, and the position of this substitution is somewhat analogous to the substitution examined by Filligame in *E. coli* (personal communication). The substitution we infer from this mutation in the mtDNA would replace aspartic acid with asparagine in a critical portion of the F_O molecule.

This portion of the molecule forms a polar pocket through which the protons flow. This pocket spans the membrane and is surrounded by the membrane lipid. Having a polar amino acid instead of an acidic amino acid at this spot results in a reduction in ATP synthesis efficiency or a slip as defined by Brown *et al.* (125–127). When this occurs there is a reduction in the capture of the energy generated by the respiratory chain, and less ATP is produced.

Additional studies by Herrnstadt (unpublished observations) confirmed the above point mutation and have provided evidence of an additional mutation at position 8289. This mutation occurs further along the subunit and comprises part of the anchor for the protein in the inner mitochondrial membrane. The mutation calls for the substitution of threonine for the normal serine at this position. Such a substitution in an amino acid loop would, at that point, turn the loop into a pleated sheet. The sheet would then continue and serve as a kink in the structure that would otherwise be quite flexible or mobile within the membrane. As such, one might surmise that the mobility of the subunit within the membrane might be affected. Indeed, studies of the functionality of the subunit within membranes varying in fluidity due to diet-induced changes in the membrane fatty acids revealed that when the membrane was more rigid, ATP synthesis was appreciably reduced and vice versa (121). Feeding hydrogenated coconut oil, corn oil, or menhaden oil has been shown to affect fluidity (121). Sprague-Dawley rats, evaluated at the same time and fed the same diets did not show such a response. Furthermore, the ability of the mitochondria to sequester and then extrude the calcium ion as part of OXPHOS is affected by the mutations in the ATPase 6 gene in the BHE/Cdb rat (128, 129). Calcium movement depends not only on the efficiency of ATP synthesis (calcium extrusion is an ATP-dependent process) but also on the degree of flexibility of the membrane-embedded portions of the ATPase complex (130). Having a base substitution in that portion of the molecule that serves as the membrane anchor limits the flexibility of the complex upon which calcium and ATP extrusion depends. Exposure of mitochondria from BHE/Cdb and Sprague-Dawley rats to varying levels of calcium showed that the mitochondria from BHE/Cdb rats were less able to extrude excess calcium and thus, this calcium became toxic at lower levels in these rats than in control rats (129).

If there is a reduction in the amount of ATP available for cell functions that are ATP dependent, some of those cell functions will be impaired. Insulin synthesis and release as discussed earlier, would be affected. The process operates at subnormal rates in NIDDM. In NIDDM-prone BHE/Cdb rats, ATP synthesis efficiency is reduced (115, 131), and so too is normal islet function (132, 133). In studies of islet cells in culture (133), the adaptive response to 7-day culture in 10 mmol/l glucose was reduced in the islets from BHE/Cdb rats. The dose response curve for glucose stimulation showed a shift to the left such that the threshold was lowered to 2.5 mmol/l. This contrasted with the results from islets of normal rats, which adapted to high glucose in an expected manner, with a threshold between 5 and 7 mmol/l.

Another tissue that has a relatively high requirement for ATP is the kidney. The kidneys use about 10% of all of the energy needed (or produced) by the body; hence, one might expect to observe lesions (or certainly renal malfunction) in rats having a defect in oxidative phosphorylation. Indeed, as noted in the initial paragraph describing these rats, renal disease is the chief cause of death. It is suspected that the lesion development in the renal tissue occurs simultaneously with the gradual decline in pancreatic islet function. Longevity studies (109, 111) have documented this simultaneous decline in function.

Lastly, just as mitochondrial mutations that associate with NIDDM in the human are maternally inherited, so too does this occur in the BHE/Cdb rat (104, 131). Two generational studies as well as the breeding records show this inheritance pattern. BHE/Cdb rats crossed with Sprague-Dawley rats yield F_1 hybrids that have the same glucose tolerance (or intolerance) and ATP production efficiency (or inefficiency) as their mothers (131). Those rats that have the BHE/Cdb maternal trait had impaired glucose tolerance and impaired mitochondrial ATP production whereas those possessing the Sprague-Dawley maternal trait did not.

How can we connect a rather minor mtDNA mutation with an initial and subtle reduction in ATP synthesis to age-related impairment in glucose tolerance and renal function? Here is a very minor gene deficit, yet, over time, this defect has major consequences. One could accept this minor defect as the cause of the age-related problems if one realizes and appreciates the degree to which the living body can accommodate and compensate for such a minor problem given a stress-free, pathogen-free environment. In the instance of the BHE/Cdb rat, we have discovered that one of the compensations that occurs is an increase in mitochondrial ATP production *via* the adenylate kinase reaction (129, 131). However, with age this compensatory mechanism doubtless begins to fail (115), and decompensation begins. We have also discovered that nutrient stress as in the use of sugar-rich, fat-rich diets also hastens decompensation and shortens the time frame for NIDDM development. The fat-rich diets might provide an additional stress by furnishing sources of DNA-damaging free radicals (111). What mechanism could explain all of these interrelated observations? In Figure 2 we propose a cascade of events that, over time, could result in NIDDM and renal dysfunction. We base this proposal on the work conducted to date on this rat strain and suggest that perhaps a similar sequence might occur in the untreated, undiagnosed human. The cascade begins with a minor reduction in ATP synthesis efficiency and over time develops such that major compromises in intermediary metabolism can be observed, which in turn serve to magnify the consequences of the genetic defect. Compromised renal function has been noted in the BHE/Cdb rat as have disturbances in intermediary metabolism and changes with age in pancreatic islet function.

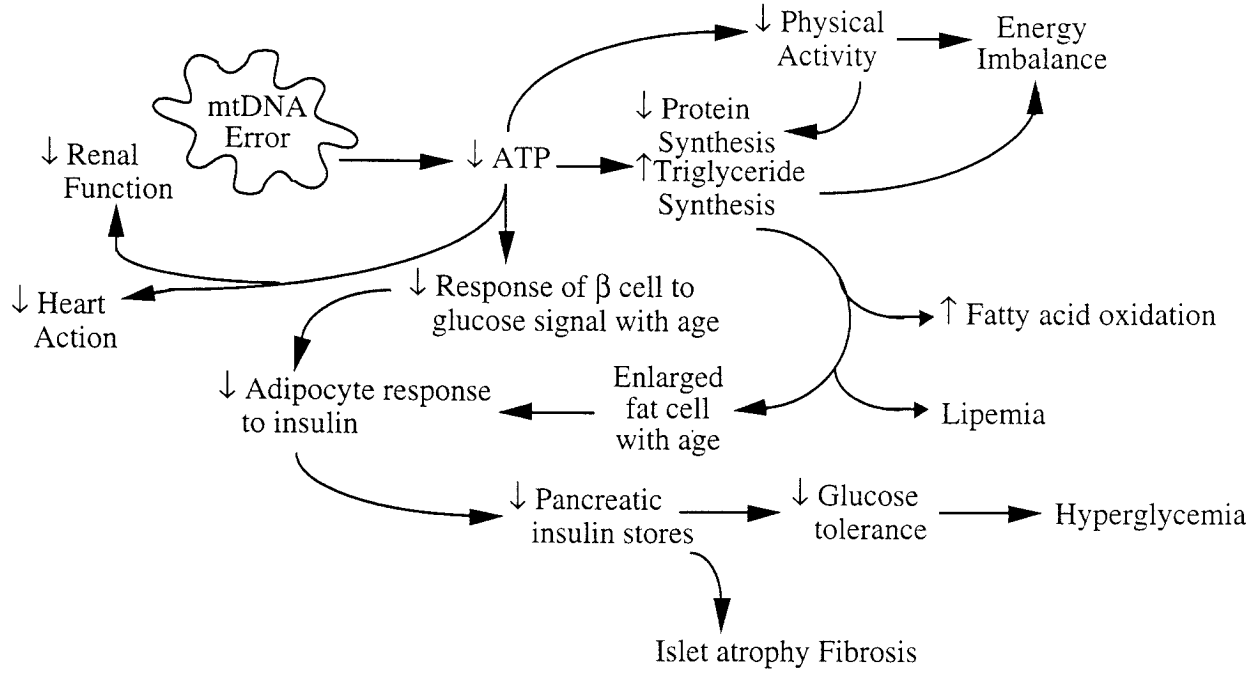

Figure 2. Proposed scheme whereby a small error can ultimately have large consequences over a lifetime.

Conclusion

This review focused on NIDDM as a phenotype for specific mitochondrial genotypes. Both humans and rats that mimic humans with NIDDM in the absence of obesity and other nuclear DNA mutations were described. The explosion of literature that has presented a bewildering array of clinical features attributable to mtDNA point mutations, deletions, additions, and rearrangements can confuse the reader. Schon *et al.* in a recent review (134) divided these features into those that are mitochondrion-selective and those that are not. Mitochondrial dysfunction influences a number of postmitotic cell types that have high oxygen and energy requirements. Included are the endocrine organs, nerve, muscle, eye, and kidney. Tissues such as the liver, while dependent on normal OXPHOS function, are able to circumvent (to some extent) the problem created by the mutation. Thus, major defects in mtDNA are manifested in high oxygen-energy dependent tissues whereas minor defects (or a low percentage of defective DNA) will show up as deviations in metabolic control mechanisms in low oxygen-energy dependent tissues. Such has been described in the BHE/Cdb rat. CNS function is adequate in these rats despite the base substitutions in the ATPase 6 gene that affect F_1F_OATPase function. On the other hand, renal, hepatic, and islet function becomes abnormal with age. These animals are homoplasmic in their ATPase 6 mutations. Humans seldom are homoplasmic with respect to mtDNA mutations that associate with NIDDM and NIDDM alone. Whereas the BHE/Cdb rat has been studied extensively, its usefulness as a tool to develop appropriate pharmaceuticals and nutriceuticals for the treatment of mt diabetes in humans has not been explored. With such a mutant animal in hand, numerous experiments can be designed to test numerous hypothesis about the roles of age, diet, and drugs in the pathophysiology of NIDDM.

Future work will no doubt include such explorations in addition to work designed to elucidate the mechanisms involved in the development of the associated renal and cardiovascular pathology. These areas have been barely touched. Through the study of these rats, we may gain important insights into the pathophysiology of mitochondrial diabetes mellitus and its treatment in the human.

1. Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mtDNA. Proc Natl Acad Sci U S A **77:**6715–6719, 1980.
2. Wallace DC. Diseases of the mtDNA. Annu Rev Biochem **61:**1175–1212, 1992.
3. Tzagoloff A, Myers AM. Biogenesis of mitochondrial genetics. Annu Rev Biochem **55:**249–285, 1986.
4. Anderson S, Bankier AT, Barrell BG, Bruijin MHL, Coulsen AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. Nature **290:**457–465, 1981.
5. Cantatore P, Attardi G. Mapping the nascent light and heavy strand transcripts on the physical map of HeLa cell mtDNA. Nucleic Acids Res **8:**2605–2625, 1980.
6. Chang DD, Clayton DA. Precise identification of individual promoters for transcription of each strand of human mtDNA. Cell **36:**635–643, 1984.
7. Mignotte F, Gueride A, Champagne AM, Mounolou JC. Direct repeats in the noncoding region of rabbit mtDNA: Involvement in the generation of intra- and inter-individual heterogeneity. Eur J Biochem **194:**561–571, 1990.
8. Monnat RJ, Reay DT. Nucleotide sequence identity of mitochondrial DNA from different human tissues. Gene **43:**205–211, 1986.
9. Shoffner JM, Wallace DC. Oxidative phosphorylation disease and mitochondrial DNA mutations: Diagnosis and treatment. Annu Rev Nutr **14:**535–568, 1994.
10. Walker JE, Gay NJ, Powell SJ, Kostina M, Dyer MR. ATP synthase from bovine mitochondria: Sequences of imported precursors of oli-

gomycin sensitivity conferral protein, factor 6, and adenosinetriphosphatase inhibitor protein. Biochemistry **26:**8613–8619, 1987.
11. Walker JE, Runswick MJ, Poulter L. ATP synthase from bovine mitochondria: The characterization and sequence analysis of two membrane-associated subunits and of corresponding cDNAs. J Mol Biol **197:**89–100, 1987.
12. Britten RJ, Kohne DE. Repeated sequences in DNA: Hundreds of thousands of copies of DNA sequences have been incorporated into the genomes of higher organisms. Science **161:**529–540, 1968.
13. Hixson JE, Clayton A. Initiation of transcription from each of the two human mitochondrial promoters requires unique nucleotide as transcriptional start sites. Proc Natl Acad Sci U S A **82:**2660–2664, 1985.
14. Gadaleta G, Pepe G, DeCandia G, Quagliariello C, Sbissá E, Saccone C. The complete nucleotide sequence of the *Rattus norvegicus* mitochondrial genome: Cryptic signals revealed by comparative analysis between vertebrates. J Mol Evol **28:**497–516, 1989.
15. Brown WM, George MM, Wilson AC. Rapid evolution of animal mtDNA. Proc Natl Acad Sci U S A **76:**1967–1971, 1979.
16. Foran DR, Hixson JE, Brown WM. Comparisons of ape and human sequences that regulate mtDNA transcription and D-loop DNA synthesis. Nucleic Acid Res **16:**5841–5861, 1988.
17. Attardi G. Animal mitochondrial DNA: An extreme example of genetic economy. Int Rev Cytol **93:**93–145, 1985.
18. Clayton DA. Replication and transcription of vertebrate mitochondrial DNA. Annu Rev Cell Biol **7:**453–478, 1991.
19. Grosjean HJ, De Henau S, Crothers DM. On the physical basis of ambiguity in genetic coding interactions. Proc Natl Acad Sci U S A **75:**610, 1978.
20. Crick FHC. Codon-anticodon pairing: The wobble hypothesis. J Mol Biol **19:**548, 1966.
21. Lewin B. Transfer RNA: The translational adaptor. In: Genes IV. Cambridge, MA: Cell Press, pp 137–170, 1990.
22. Barrell BG, Bankier AT, Drouin J. A different genetic code in human mitochondria. Nature **282:**189–194, 1979.
23. Maréchal-Douard L, Dietrich A, Wiel JH. Adaptation of tRNA population to codon usage in cellular organelles. In: Hatfield D, Lee B, Pirthe H, Eds. Transfer RNA in Protein Synthesis. Boca Raton, FL: CRC Press, pp 125–140, 1992.
24. Muller PP, Reif MK, Zoughou S, Sengstag C, Mason TL, Fox TD. A nuclear mutation that post-transcriptionally blocks accumulation of a yeast mitochondrial gene product can be suppressed by a mitochondrial gene rearrangement. J Mol Biol **174:**431–452, 1984.
25. Dieckmann CL, Homison G, Tzagoloff AJ. Assembly of the mitochondrial membrane system: Nuclear sequence of yeast gene (CBP1) involved in 5′ processing of cytochrome b pre-mRNA. J Biol Chem **259:**4732–4738, 1984.
26. McGraw P, Tzagoloff A. Assembly of the mitochondrial membrane system: Characterization of a yeast nuclear gene involved in processing of cytochrome b pre-mRNA. J Biol Chem **258:**9459–9468, 1983.
27. Pillar T, Lang BF, Steinberger I, Vogt B, Kaudewitz F. Expression of the "split-gene" cob in yeast mtDNA: Nuclear mutations specifically block the excision of different introns from its primary transcript. J Biol Chem **258:**7954–7959, 1983.
28. Dieckmann CL, Tzagoloff AJ. Assembly of the mitochondrial membrane system: CBP6, a yeast nuclear gene necessary for synthesis of cytochrome b. J Biol Chem **260:**1513–1520, 1985.
29. Schatz G, Groot GS, Mason T, Rouslin W, Wharton DC, Salitzgaber J. Biogenesis of mitochondrial inner membrane in bakers' yeast. Fed Proc **31:**21–29, 1972.
30. Montoya J, Perez-Martos A, Garstka HL, Wiesner RJ. Regulation of mitochondrial transcription by mitochondrial transcription factor A. Mol Cell Biochem **174:**227–230, 1997.
31. Demonacos CV, Karayanni N, Hatzoglou E, Tsiriyiotis C, Spandidos DA. Mitochondrial genes as sites of primary action of steroid hormones. Steroids **61:**226–232, 1996.
32. Degoul F, Nelson I, Amselem S, Romero N, Obermaier-Kusser B, Ponsot G, Marsac C, Lestienne P. Different mechanisms inferred from sequences of human mitochondrial DNA deletions in ocular myopathies. Nucleic Acids Res **19:**493–496, 1990.
33. Zeviani M, Gellera C, Antozzi C, Rimoldi M, Morandi L, Villani F, Tiranti V, DiDonato S. Maternally inherited myopathy and cardiomyopathy: Association with mutation in mitochondrial DNA tRNA(Leu)(UUR). Lancet **338:**143–147, 1991.
34. Buroker NE, Brown JR, Gilbert TA, O'Hara PJ, Beckenback AT, Thomas WK, Smith MJ. Length heteroplasmy of sturgeon mtDNA: An illegitimate elongation model. Genetics **124:**157–163, 1991.
35. Madsen CS, Ghivizzani SC, Hauswirth WW. *In vivo* and *in vitro* evidence for slipped mispairing in mammalian mitochondria. Proc Natl Acad Sci U S A **90:**7671–7675, 1993.
36. Corral-Debrinski M, Stepien G, Shoffner JM, Lott MT, Kanter K, Wallace DC. Hypoxemia is associated with mtDNA damage and gene induction. Implications for cardiac disease. JAMA **266:**1812–1816, 1991.
37. Wei Y-H, Kao S-H. Mitochondrial DNA mutations and lipid peroxidation in human aging. In: *Nutrients and Gene Expression* (Berdanier CD, ed). Boca Raton, FL: CRC Press, pp 165–188, 1996.
38. Yen TC, Pang CY, Hsieh RH, Su CH, King KL, Wei YH. Age-dependent 6-kb deletion in human mitochondrial DNA. Biochem Mol Biol Int **26:**457–468, 1992.
39. Yen TC, Su HH, King KL, Wei YH. Aging-associated 5-kb deletion in human liver mitochondrial DNA. Biochem Biophys Res Commun **178:**124–131, 1991.
40. Lestienne P. Mitochondrial DNA mutations in human diseases: A review. Biochimie **74:**123–130, 1992.
41. Zeviani M, Bresolin N, Gellera C, Bordoni A, Pannacci M, Amati P, Moggio M, Servidei S, Scarlato G, DiDonato S. Nucleus-driven multiple large-scale deletions of the human mitochondrial genome: A new autosomal dominant disease. Am J Hum Genet **47:**904–914, 1990.
42. Ghivizzani SC, Mackay SD, Madsen CS, Laipis PJ, Hauswirth WW. Transcribed heteroplasmic repeated sequences in porcine mtDNA D-loop region. J Mol Evol **137:**36–47, 1993.
43. Simonetti S, Chen X, DiMauro S, Schon EA. Accumulation of deletions in human mitochondria during normal aging: Analysis by quantitative PCR. Biochem Biophys Acta **1180:**113–122, 1992.
44. Asano K, Nakamura M, Sato T, Tauchi H, Asano A. Age dependency of mtDNA decrease differs in different tissues of rat. J Biochem **114:**303–306, 1993.
45. Calleja M, Pena P, Ugalde C, Ferreiro C, Marco R, Garesse R. Mitochondrial DNA remains intact during Drosophila aging, but the levels of mitochondrial transcripts are significantly reduced. J Biol Chem **268:**1891–1898, 1993.
46. Gerbitz KD. Does the mitochondrial DNA play a role in the pathogenesis of diabetes? Diabetologia **35:**1181–1186, 1992.
47. Gerbitz KD, Gempel K, Brdiczka D. Mitochondria and diabetes: Genetic, biochemical, and clinical implications of the cellular energy circuit. Diabetes **45:**113–126, 1996.
48. Gerbitz KD, Paprotta A, Jaksch M, Zierz S, Drechsel J. Diabetes mellitus is one of the heterogeneous phenotypic features of a mtDNA point mutation within the tRNA leu gene. FEBS Lett **321:**194–196, 1993.
49. Reardon W, Ross RJ, Sweeney MG, Luxon LM, Pembry ME, Harding AE, Trembath RC. Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. Lancet **340:**1376–1379, 1992.
50. Wakeley J. Substitution rate variation among sites in hypervariable region 1 of human mtDNA. J Mol Evol **37:**613–623, 1993.
51. Howell N, McCullough DA, Kubacka I, Halvorson S, Mackey D. The sequence of human mtDNA: The question of errors versus polymorphisms. Am J Human Genet **50:**1333–1337, 1992.
52. Newman B, Shelby JV, King MC, Selmenda C, Fabsitz R, Friedman GD. Concordance for type 2 (noninsulin-dependent) diabetes mellitus in male twins. Diabetologia **30:**763–768, 1987.
53. Vanheim CM, Rotter JI. Genetics of diabetes mellitus. In: Alberti KGMM, Defronzo RA, Keen H, Zimmlet P, Chichester T, Eds. International Textbook of Diabetes Mellitus. New York: Wiley, pp 31–98, 1992.
54. Stoffel M, Froguel PH, Takeda J, Zouali H, Vionnet N, Nishi S, Weber IT, Harrison RW, Pilkis SJ, Lesage S, Vaxillaire M, Velho G, Sun F, Iris F, Passa PH, Cohen D, Bell GI. Human glucokinase gene: Isolation, characterization and identification of two missense mutations linked to early-onset noninsulin-dependent (type 2) diabetes mellitus. Proc Natl Acad Sci U S A **89:**7698–7702, 1992.
55. Bell GI, Froguel P, Nishi S, Pilkis SJ, Stoffel M, Takeda J, Vionnet N, Yasuda K. Mutations in the human glucokinase gene and diabetes mellitus. Trends Endocrinol Metab **4:**86–90, 1993.
56. Chiu KC, Tanizawa Y, Permutt MA. Glucokinase gene variants in the common form of NIDDM. Diabetes **42:**579–582, 1993.

57. Matschinsky FM, Liang Y, Keavan P, Wang L, Froguel P, Velho G, Cohen D, Permutt MA, Tanizawa Y, Jetton TL, Niswender K, Magnuson MA. Glucokinase as pancreatic β-cell, glucose sensor and diabetes gene. J Clin Invest **2:**2092–2098, 1993.
58. Velho G, Blanche H, Vaxillaire M, Bellanne-Chantelot C, Pardini VC, Timsit J, Passa P, Deschamps I, Robert JJ, Weber IT, Marotta D, Pilkis SJ, Lipkind GM, Bell GI, Froguel P. Identification of 14 new glucokinase mutations and description of the clinical profile of 42 MODY-2 families. Diabetologia **40:**217–224, 1997.
59. Awata T, Kurihara S, Kikuchi C, Takai S-I, Inoue I, Ishii C, Takahashi K, Negishi K, Yoshida Y, Hagura R, Kanazawa Y, Katayama S. Evidence for association between the class 1 subset of the insulin gene minisatellite (IDDM 2 locus) and IDDM in the Japanese population. Diabetes **46:**1637–1642, 1997.
60. Kahn SE, Halban PA. Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. Diabetes **46:**1725–1732, 1997.
61. Dreyer M, Matthaei S, Kuhnau J, Rudiger HW. Prolonged plasma half-life of insulin in patients with a genetic defect of high affinity binding sites. Horm Metab Res **18:**247–249, 1986.
62. Livnat TM, Chen-Zion M, Beitner R. Uncoupling of mitochondrial oxidative phosphorylation abolishes the stimulatory action of insulin on the binding of glycolytic enzymes to muscle cytoskeleton. Int J Biochem **25:**993–997, 1993.
63. Civelek VN, Deeney JT, Shalosky NJ, Tornheim K, Hansford RG, Prentki M, Corkey BE. Regulation of pancreatic beta-cell mitochondrial metabolism: Influence of Ca^{2+}, substrate, and ADP. Biochem J **318:**615–621, 1996.
64. Nichols CG, Shyng SL, Nestorowicz A, Glaser B, Clement JP IV, Gonzalez G, Aguilar-Bryan L, Permutt MA, Bryan J. Adenosine diphosphate as an intracellular regulator of insulin secretion. Science **272:**1785–1787, 1996.
65. Nilsson T, Schultz V, Berggren P-O, Corkey B, Tornhein K. Temporal pattern of changes in ATP/ADP ratio, glucose-6-phosphate and cytoplasmic free Ca^{2+} in glucose-stimulated pancreatic β cells. Biochem J **314:**91–94, 1996.
66. Isshiki G. Impaired glucose tolerance in the young. Nippon Rinsho **54:**2766–2772, 1997.
67. Alcolado JC, Alcolado R. Importance of maternal history of noninsulin-dependent diabetic patients. BMJ **302:**1178–1180, 1991.
68. Alcolado JC, Majid A, Brockington M, Sweeney MG, Morgan R, Rees A, Harding AE, Barnett AH. Mitochondrial gene defects in patients with NIDDM. Diabetologia **37:**372–376, 1994.
69. Alcolado JC, Thomas AW. Maternally inherited diabetes mellitus: The role of mitochondrial DNA defects. Diabet Med **12:**102–108, 1995.
70. Suzuki S. Clinical characterization of diabetes mellitus in the families with mitochondrial encephalomyopathies. Nippon Rinsho **52:**2606–2610, 1994.
71. Suzuki S, Hinokio Y, Hirai S, Onoda M, Matsumoto M, Ohtomo M, Kawasaki H, Satoh Y, Akai H, Abe K. Diabetes with mitochondrial gene $tRNA^{Lys}$ mutation. Diabetes Care **17:**1428–1432, 1994.
72. Suzuki Y, Tsukuda K, Atsumi Y, Goto Y, Hosokawa K, Asahina T, Nonaka I, Matsuoka K, Oka Y. Clinical picture of a case of diabetes with mitochondrial tRNA mutation at position 3271. Diabetes Care **19:**1304–1305, 1996.
73. Hinokio Y, Suzuki S, Komatu K, Ohtomo M, Onoda M, Matsumoto M, Hirai S, Sato Y, Akai H, Abe K, Miyabayasi S, Abe R, Toyoto T. A new mitochondrial DNA deletion associated with diabetic amyotrophy, diabetic myoatrophy and diabetic fatty liver. Muscle Nerve **3:**S142–S149, 1995.
74. Walker M, Taylor RW, Stewart MW, Bindoff LA, Jackson MJ, Alberti GK, Turnbull DM. Insulin sensitivity and mitochondrial gene mutation. Diabetes Care **18:**273–275, 1995.
75. Piccolo G, Aschei M, Ricordi A, Banfi P, Lo Curto F, Fratino P. Normal insulin receptors in mitochondrial myopathies with ophthalmoplegia. J Neurol Sci **94:**163–172, 1989.
76. Gebhart SS, Shoffner JM, Koontz D, Kaufman A, Wallace D. Insulin resistance associated with maternally inherited diabetes and deafness. Metabolism **45:**526–531, 1996.
77. Oka Y, Katagiri H, Ishihara H, Asano T, Kobayashi T, Kikuchi M. β-cell loss and glucose-induced signaling defects in diabetes mellitus caused by mitochondrial tRNA(Leu(UUR)) gene mutation. Diabet Med **13:**S98–S102, 1996.
78. Matschinsky FM. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes **45:**223–241, 1996.
79. Moraes CT, Ciacci F, Bonilla E, Jansen C, Hirano M, Rao N, Locelace RE, Rowland LP, Schon EA, DiMauro S. Two novel pathogenic mitochondrial DNA mutations affecting organelle number and protein synthesis: Is the tRNA(Leu(UUR)) gene an etiologic hot spot? J Clin Invest **92:**2909–2915, 1993.
80. Morten KJ, Cooper JM, Brown GK, Lake BD, Pike D, Poulton J. A new point mutation associated with mitochondrial encephalomyopathy. Hum Mol Genet **2:**2081–2087, 1993.
81. Lempkes HHPJ, de Vijlder M, Struyvenberg PAA, van de Kamp JJP, Frolich M. Maternal inherited diabetes deafness of the young (MIDDY), a new mitochondrial syndrome. Diabetologia **32:**509A, 1989.
82. van der Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PAA, van de Kamp JJP, Maassen JA. Mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet **1:**368–371, 1992.
83. Velho G, Byrne MM, Clement K, Sturis J, Pueyo ME, Blanche H, Vionnet N, Fiet J, Passa P, Robert JJ, Polonsky KS, Froguel P. Clinical phenotypes, insulin secretion, and insulin sensitivity in kindreds with maternally inherited diabetes and deafness due to mitochondrial $tRNA^{Leu(UUR)}$ gene mutation. Diabetes **45:**478–487, 1996.
84. Quade A, Zierz S, Klingmuller D. Endocrine abnormalities in mitochondrial myopathy with external ophthalmoplegia. Clin Invest **70:**396–402, 1992.
85. Dunbar DR, Moonie PA, Swingler RJ, Davidson D, Roberts R, Holt IJ. Maternally transmitted partial direct tandem duplication of mitochondrial DNA associated with diabetes mellitus. Hum Mol Genet **2:**1619–1624, 1993.
86. Poulton J, Deadman ME, Gardiner RM. Duplications of mitochondrial DNA in mitochondrial myopathy. Lancet **1:**236–240, 1989.
87. Poulton J, Morten KJ, Weber K, Brown GK, Bindoff L. Are duplications of mitochondrial DNA characteristic of Kearns-Sayre syndrome? Hum Mol Genet **3:**947–951, 1994.
88. Poulton J, O'Rahilly S, Morten KJ, Clark A. Mitochondrial DNA, diabetes, and pancreatic pathology in Kearns-Sayre syndrome. Diabetologia **38:**868–871, 1995.
89. Rotig A, Bessis JL, Romero N, Cormier V, Saudubray JM, Narcy P, Lenoir G, Rustin P, Munnich A. Maternally inherited duplication of the mitochondrial genome in a syndrome of proximal tubulopathy, diabetes mellitus, and cerebellar ataxia. Am J Hum Genet **50:**364–370, 1992.
90. Majander A, Suomaleinen A, Vettebranta K, Sarioly H, Pekkio M, Holmberg C, Pihko H. Congenital hypoplastic anemia, diabetes, and severe renal tubular dysfunction associated with a mitochondrial DNA deletion. Pediatr Res **30:**327–330, 1991.
91. Superti-Furga A, Schoenle E, Tuchschmid P, Caduff R, Sabato V, DeMattia D, Gitzelmann R, Steinmann B. Pearson bone marrow–pancreas syndrome with insulin-dependent diabetes, progressive renal tubulopathy, organic aciduria, and elevated fetal haemoglobin caused by deletion and duplication of mitochondrial DNA. Eur J Pediatr **152:**44–50, 1993.
92. Lankin M, Locke S. Progressive ocular myopathy with ovarian insufficiency and diabetes mellitus: Report of a case. Diabetes **10:**228–231, 1961.
93. Mechler F, Fawcett PRW, Mastaglia FL, Hudgson P. Mitochondrial myopathy: A study of clinically affected and asymptomatic members of a six-generation family. J Neurol Sci **50:**191–200, 1981.
94. Goto Y, Nonaka I. Mitochondrial encephalomyopathies: 3243 mutation as a central matter. Rinsho Shinkeigaku **35:**1425–1426, 1995.
95. Schulz JB, Klockgether T, Dichgans J, Seibel P, Reichmann H. Mitochondrial gene mutations and diabetes mellitus. Lancet **341:**438–449, 1993.
96. Sue CM, Holmes-Walker DJ, Morris JGL, Bovages SC, Crimmins DS, Byrne E. Mitochondrial gene mutations and diabetes mellitus. Lancet **341:**437–438, 1993.
97. Ballinger SW, Shoffner JM, Hedaya EV, Trounce I, Polak MA, Koontz DA, Wallace DC. Maternally transmitted diabetes and deafness associated with a 10.4-kb mitochondrial DNA deletion. Nat Genet **1:**11–15, 1992.
98. Ballinger SW, Shoffner JM, Gebhart S, Koontz DA, Wallace DC. Mitochondrial diabetes revisted. Nat Genet **7:**458–459, 1994.

99. Takeda N, Tanamura A, Iwai T, Nakamura I, Kato M, Ohkuba T, Noma K. Mitochondrial DNA deletion in human myocardium. Mol Cell Biochem **119:**105–108, 1993.
100. Mathews CE, McGraw RA, Berdanier CD. A point mutation in the mitochondrial DNA of diabetes prone BHE/Cdb rats. FASEB J **9:**1638–1642, 1995.
101. Anderson C, Herrnstadt C, Miller C, Olefsky J, Davis R. Mutations in mitochondrial ATPase 8 gene are associated with NIDDM. Diabetes **46:**174A, 1997.
102. Mutvei A, Nelson BD. Energy requirements for protein synthesis in isolated rat liver mitochondria. Biochem Biophys Res Comm **105:**916–921, 1982.
103. Malaisse WJ. Glucose sensing by the pancreatic β cell. The mitochondrial part. Int J Biochem **24:**693–701, 1992.
104. Mathews CE, Berdanier CD. Inheritance of a mitochondrial DNA defect and impaired glucose tolerance in BHE/Cdb rats. Diabetologia (in press).
105. Grosskopf R, Feldmann H. Analysis of a DNA segment from rat liver mitochondria containing genes for the cytochrome oxidase subunits I, II, and ATPase 6 and several tRNA genes. Curr Genet **4:**151–158, 1981.
106. Berdanier CD. The BHE rat: An animal model for the study of noninsulin-dependent diabetes mellitus. FASEB J **5:**2139–2144, 1991.
107. Berdanier CD. Diet can influence mitochondrial respiration in genetically diabetic BHE/Cdb rats: A review. Res Trends Endocrinol **12:**103–115, 1993.
108. Berdanier CD. Noninsulin-dependent diabetes in the non-obese BHE/Cdb rat. In: Shafrir E, Ed. Recent Advances in Animal Diabetes. London: Smith Gordon Publishers, pp 231–246, 1994.
109. Berdanier Cd, Johnson B, Hartle DK, Crowell WA. Lifespan is shortened in BHE/Cdb rats fed a diet containing 9% menhaden oil and 1% corn oil. J Nutr **122:**1109–1131, 1992.
110. Berdanier CD, Kras KM, Wickwire K, Hall DG. Whole egg delays the age-related impaired glucose tolerance of BHE/Cdb rats. Proc Soc Exp Biol Med **219:**28–36, 1998.
111. Berdanier CD, Kras KM, Hall DG, Gunnett C, Hartle D. Progressive glucose intolerance and renal disease in aging rats. Int J Diabetes **5:**27–38, 1997.
112. Lakshmanan MR, Berdanier CD, Veech RL. Comarative studies on lipogenesis and cholesterogenesis in lipemic BHE rats and normal Wistar rats. Arch Biochem Biophys **183:**355–360, 1977.
113. Berdanier CD, Tobin RB, DeVore V, Wurdeman R. Studies on the metabolism of glycerol by hyperlipemic and normolipemic rats. Proc Soc Exp Biol Med **157:**5–11, 1978.
114. Berdanier CD. Rat strain differences in gluconeogenesis by isolated hepatocytes. Proc Soc Exp Biol Med **169:**74–79, 1982.
115. Berdanier CD, Thomson AR. Comparative studies on mitochondrial respiration in four strains of rats *Rattus norvegicus.* Comp Biochem Physiol **85B:**531–535, 1986.
116. Fowler K, Berdanier CD. Low-dose intakes of mitochondrial uncouplers differentially affect hepatic lipogenesis in BHE/Cdb and Sprague-Dawley rats. Biochemistry Archives **7:**269–274, 1991.
117. Wander RC, Berdanier CD. Effects of type of dietary fat and carbohydrate on gluconeogenesis in isolated hepatocytes from BHE rats. J Nutr **116:**1156–1164, 1986.
118. Berdanier CD, Kim M-J C. Hyperthyroidism does not induce an increase in mitochondrial respiration. J Nutr Biochem **4:**10–19, 1993.
119. Deaver OE Jr., Wander RC, McCusker RH, Berdanier CD. Diet effects on membrane phospholipid fatty acids and mitochondrial function in BHE rats. J Nutr **116:**1148–1155, 1986.
120. Kim MC, Berdanier CD. Glucose homeostasis in thyroxine-treated BHE/Cdb rats fed corn oil or hydrogenated coconut oil. J Nutr Biochem **4:**10–19, 1993.
121. Kim MC, Berdanier CD. Nutrient-gene interactions determine mitochondrial function: Effect of dietary fat. FASEB J **12:**112–118, 1998.
122. Hood DA, Simoneau J-A, Kelly AM, Pette D. Effect of thyroid status on the expression of metabolic enzymes during chronic stimulation. Am J Physiol **263:**C788–C793, 1992.
123. Soboll S. Thyroid hormone action on mitochondrial energy transfer. Biochim Biophys Acta **1144:**1–16, 1993.
124. Van Itallie CM. Thyroid hormone and dexamethasone increase the levels of a messenger ribonucleic acid for a mitochondrially encoded subunit but not for a nuclear-encoded subunit of cytochrome-*c* oxidase. Endocrinology **127:**55–62, 1990.
125. Brown GC. Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem J **284:**1–13, 1992.
126. Brown GC, Lakin-Thomas PL, Brand MD. Control of respiration and oxidative phosphorylation in isolated rat liver cells. Eur J Biochem **192:**355–362, 1990.
127. Hafner RP, Brown GC, Brand MD. Analysis of the control of respiration rate, phosphorylation rate, proton leak rate, and protonmotive force in isolated mitochondria using the "top-down" approach of metabolic control theory. Eur J Biochem **188:**313–319, 1990.
128. Kim S-B. Defects in hepatic mitochondrial F_1F_OATPase of BHE/Cdb rats. PhD dissertation (Berdanier CD, Director), University of Georgia, 1997.
129. Kim S-B, Berdanier CD. Further studies on oxidative phosphorylation in diabetes-prone BHE/Cdb rats: Increased sensitivity to the calcium ion. J Nutr Biochem (in press).
130. Pedersen PL. Frontiers in ATP synthase research: Understanding the relationship between subunit movements and ATP synthesis. J Bioenerg Biomembr **28:**389–395, 1996.
131. Mathews CE. Inheritance of NIDDM in the BHE/Cdb rat. PhD dissertation (Berdanier CD, Director), University of Georgia, 1997.
132. Liang Y, Bonner-Weir S, Wu Y-J, Berdanier CD, Berner DK, Efrat S, Matschinsky F. *In situ* glucose uptake and glucokinase activity of pancreatic islets in diabetic and obese rodents. J Clin Invest **93:**2473–2481, 1994.
133. Collins H, Li G, Najafi H, Doliba N, Berdanier CD, Matschinsky FM. Early manifestation of pancreatic islet dysfunction in the BHE/Cdb rat, a model of type II diabetes caused by a genetic mitochondrial defect. Diabetes **47:**A58, 1998.
134. Schon EA, Bonilla E, DeMauro S. Mitochondrial DNA mutations and pathogenesis. J. Bioenerg Biomembr **29:**131–149, 1997.