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SYMPOSIUM: Mitochondrial Encephalomyopathies

Mitochondrial Respiratory Chain Diseases and Mutations in Nuclear DNA: A Promising Start?

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Introduction

For more than a decade, the search for pathogenic mutations in human diseases due to respiratory chain dysfunction has been focused on the mitochondrial genome. Over 100 mutations affecting both tRNA genes and genes specifying subunits of respiratory chain complexes have now been found (11, 36). In the past few years, the focus of attention has shifted to the search for mutations within the nuclear genome (nDNA), including genes that encode structural subunits of the respiratory chain, genes that are needed for the assembly of these subunits, and genes that are involved in intergenomic signalling. We will focus here on known nuclear mutations affecting specific complexes of the respiratory chain, and their assembly. Disorders involving intergenomic signalling are discussed in the review by Hirano and Vu.

The respiratory chain

The respiratory chain consists of four multisubunit complexes (Complexes I-IV) which, together with complex V (ATP synthase), form the respiratory chain/oxidative phosphorylation system. The first four complexes act together to generate a proton gradient that is coupled to the conversion of ADP and inorganic phosphate to ATP in Complex V. The respiratory chain is unique, in that it is under the control of two separate genomes: mtDNA and nDNA. Unlike nDNA, the entire sequence of the human mitochondrial genome is known (2). It is a 16,569-bp circle of double-stranded DNA containing genes specifying 2 ribosomal RNAs, 22 transfer RNAs, and 13 structural proteins; all 13 are subunits of the various respiratory chain complexes (Figure 1). Each complex of the respiratory chain also contains subunits encoded by nuclear genes, which are assembled together with the mtDNA-encoded subunits into the respective holoenzymes, located in the inner mitochondrial membrane. The coordination of the signals between the nucleus and the mitochondrion are poorly understood, and only now are beginning to be elucidated (35, 51).

Mendelian-inherited respiratory chain diseases

Diseases associated with mtDNA defects typically follow a maternal pattern of inheritance, but some are sporadic (see the review by DiMauro and Andreu). In contrast, disorders associated with nDNA follow the traditional mendelian patterns of inheritance. Even though all components of the respiratory chain contain nuclear-encoded subunits, pathogenic mutations have been identified thus far only in Complexes I, II, and IV. Mutations in these complexes were identified by adopting the strategy of sequencing the most conserved subunits in well-selected patients with isolated Complex I deficiency. Other techniques employed both a candidate gene approach and techniques that screen the entire nuclear genome (e.g. linkage analysis; microcell-mediated chromosome transfer). Despite these search methods, mutations in Complex III and V have eluded detection thus far. Whether mutations in these respiratory chain components are in fact rare (but await discovery), or are biologically so severe that they are incompatible with life, is a matter of speculation. What is clear however, is that the search for the cause of mendelian-inherited respiratory chain disorders has only just begun and will carry us well into the next developmental stage of mitochondrial medicine.

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Complex I disorders

Complex I, or nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase, reduces NADH and shuttles electrons to Coenzyme Q_{10} (CoQ_{10}). It is the largest enzyme complex of the respiratory chain and is comprised of at least 42 subunits (the exact number is unknown), of which 7 are encoded by the mitochondrial genome (40). It is therefore perhaps not surprising that isolated Complex I deficiency appears to be one of the most common causes of mitochondrial encephalomyopathies (23, 32).

Patients with Complex I deficiency usually present at birth or in early childhood with severe, often fatal, multisystemic disorders frequently dominated by brain dysfunction. The most common clinical presentation is Leigh syndrome (LS), with 40-50% of these cases having associated cardiomyopathy (23, 29, 31). Fatal neonatal lactic acidosis is also common. Less frequent presentations include hepatopathy, renal tubulopathy, exercise intolerance, and cardiomyopathy with cataracts (17, 32).

Patients with LS and complex I deficiency typically have vomiting, failure to thrive, and respiratory difficulties. Infants usually develop hypotonia and brainstem dysfunction, and, less frequently, seizures (18, 37). If children survive the neonatal period, they may develop severe psychomotor retardation and depressed tendon reflexes. Progressive neurological dysfunction usually ensues until death in early to late infancy, although one child with severe mental retardation survived into late childhood (37). Heart involvement is rare in nDNA-encoded Complex I disorders, as only one such case, with evidence of hypertrophic cardiomyopathy, has been reported (18). All nDNA-encoded Complex I deficiencies described to date have been inherited as recessive traits.

Neuroimaging in patients usually shows bilateral basal ganglia and mesencephalic lesions, consistent with LS, but on occasions, white matter involvement (37) and non-specific atrophy are present (37, 50). Arterial and CSF lactate levels may be elevated, but are often normal. Muscle histology has shown non-specific changes, such as reduced number of small type I fibers. Ragged-red fibers (RRF) have never been reported in muscle biopsies from patients with nuclear-encoded, and only rarely in patients with mtDNA-encoded, Complex I gene mutations.

Biochemical evidence of isolated Complex I deficiency is usually found in muscle or cultured skin fibroblasts of patients, although postmortem studies of one patient showed that the biochemical defect was present in all tissues examined (brain, heart and liver) (18). Conversely, Complex I deficiency can also be tissue-specific; in these cases, analysis of unaffected tissues will fail to detect a defect (32).

Even though isolated Complex I deficiency is encountered relatively frequently, pathogenic mutations have been found in only four of the 35 nuclear-encoded subunits of Complex I (see Table 1). All but one of these subunits are the human homologues of proteins found in *E. coli*, implying that these are highly conserved and important subunits probably essential for Complex I function. Point mutations in the *NDUFV1* flavoprotein gene caused a fatal leucodystrophy with, interestingly, myoclonic epilepsy (37). Mutations in three other subunits of Complex I — a 5-bp tandem duplication in the *NDUFS4* iron-sulfur protein gene (50), and point mutations in the hydrophobic protein genes *NDUFS7* (48) and *NDUFS8* (18) — all caused Leigh syndrome. The mechanisms by which the mutations cause these respiratory chain disorders is unknown.

Complex II disorders

Complex II, or succinate dehydrogenase-ubiquinone oxidoreductase, oxidizes succinate to fumarate (in the citric acid cycle) and transfers electrons from FADH2 to CoQ_{10} (in the respiratory chain). It is comprised of four subunits: the flavoprotein (Fp; subunit SDHA) and the iron-sulfur protein (Ip; subunit SDHB) make up the catalytic core, while the cytochrome b heme-protein that anchors the core to the inner mitochondrial membrane is composed of a large (cybL; subunit SDHC) and a small (cybS; subunit SDHD) cytochrome b subunit. Complex II is the only respiratory chain complex that is encoded entirely by the nuclear genome.

There is a wide clinical spectrum of disease associated with Complex II deficiency, including Kearns-Sayre syndrome (30), muscle weakness (13), hypertrophic cardiomyopathy (33), Leigh syndrome (6), optic atrophy and cerebellar ataxia (43), and hereditary paraganglioma (4). However, to date, only three pathogenic mutations in Complex II genes have been identified.

The first mutation involved two sisters with LS. Both presented with motor regression in early infancy and developed rigidity, bilateral pyramidal signs, cortical blindness, and died within a few months of disease onset (7, 43). Succinate dehydrogenase (SDH) activity was decreased in muscle, fibroblasts, and lymphocytes. Both sisters had a homozygous mutation in the Fp subunit of Complex II (i.e. *SDHA*), converting Arg-544 to Trp. Recently, another child with LS, who developed truncal ataxia in early infancy, was found to be a compound het-

Complex	Gene	mRNA	Protein	Clinical features	Ref
Complex I	NDUFS4	466ins5 (dup)	Frameshift	Leigh syndrome	50
	NDUFS7	G364A	V122M	Leigh syndrome	48
	NDUFS8	C236T	P79L	Leigh syndrome	18
		G305A	R102H	Leigh syndrome	18
	NDUFV1	C175T	R59X	Leukodystrophy/myoclonic epilepsy	37
		C1022T	T423M	Leukodystrophy/myoclonic epilepsy	37
		C1268T	A341V	Leukodystrophy/myoclonic epilepsy	37
Complex II	SDHA	A25T	M1L	Leigh syndrome	27
		C1595T	A524V	Leigh syndrome	27
		C1684T	R554W	Leigh syndrome	6
	SDHD	C117T	Q36X	Hereditary paraganglioma	4
		C123T	R38X	Hereditary paraganglioma	4
		C253T	P81L	Hereditary paraganglioma	4
		G285T	D92Y	Hereditary paraganglioma	4
		A316T	H102L	Hereditary paraganglioma	4
Complex III	None				
Complex IV	SURF1	37ins17	Frameshift	Leigh syndrome	45
		G74A	W25X	Leigh syndrome	46
		239+1G→T	Frameshift (ss)	Leigh syndrome	46
		326del10,insAT	Frameshift (ss)	Leigh syndrome	55
		337+2T→C	Frameshift (ss)	Leigh syndrome	55
		G385A	G124E	Leigh syndrome	28
		516+2T→G	Frameshift (ss)	Leigh syndrome	45
		550delAG	Frameshift	Leigh syndrome	45
		552delG	Frameshift	Leigh syndrome	46
		587insCAGG	Frameshift	Leigh syndrome	42
		588insCTGC	Frameshift	Leigh syndrome	46
		589insCTGC	Frameshift	Leigh syndrome	28
		C688T	R230X	Leigh syndrome	9
		T751C	I246T	Leigh syndrome	28
		758del2	Frameshift	Leigh syndrome	46
		C765T	Q251X	Leigh syndrome	55
		766-3C→G	Frameshift (ss)	Leigh syndrome	28
		772delCC	Frameshift	Leigh syndrome	45
		790delAG	Frameshift	Leigh syndrome	46
		T820G	Y274D	Leigh syndrome	44
		G808T*	E270X	Leigh syndrome	54
		828delCT	Frameshift	Leigh syndrome	42
		855delCT	Frameshift	Leigh syndrome	55
		868insT	Frameshift	Leigh syndrome	45
		882insT	Frameshift	Leigh syndrome	55
	SCO2	C1280T	Q53X	Infantile cardioencephalomyopathy	26
		C1391T	R90X	Infantile cardioencephalomyopathy	15
		G1541A	E140K	Infantile cardioencephalomyopathy	26
		T1575C	L151P	Infantile cardioencephalomyopathy	†
		C1634T	R171W	Infantile cardioencephalomyopathy	15
		C1797T	S225F	Infantile cardioencephalomyopathy	26
	COX10	C612A	N204K	Encephalopathy /renal tubulopathy	49
Complex V	None			Luft disease (?)	
Coenzyme Q10	None			Myopathy, myoglobinuria, seizures; cerebellar ataxia	

Table 1. *Nuclear-encoded gene mutations associated with mitochondrial disease.*
* Denoted erroneously as G822T in (54); † Our unpublished data; ss = splice site mutation.
Genbank accession numbers: *COX10* (U09466.1); *NDUFS4* (AF020351.1); *NDUFS7* (by PCR; nt+1 @ initiator Met); *NDUFS8* (U65579.1; nt+1 @ initiator Met); *NDUFV1* (AF053070.1; nt+1 @ initiator Met); *SCO2* (AF177385.1); *SDHA* (L12936.1); *SDHD* (AB006202.1); *SURF1* (Z35093.1). *SURF1* mRNA is numbered starting with the initiator Met codon either as nt+1 (references 9, 44, 45, 46, 54) or nt+15 (references 28, 42, 55).

erozygote for mutations within the Fp gene, at amino acid positions 1 (converting Met to Leu) and 524 (converting Ala to Val) (27).

Mutations in the *SDHD* gene that encodes the cybS protein have been identified in patients with hereditary paraganglioma. This is a rare autosomal dominant disorder associated with a genomically imprinted locus on chromosome 11 (with incomplete penetrance when transmitted through fathers, but no expression of the disease when transmitted through mothers). It is characterized by the development of benign vascularized tumors in the head and neck, most commonly in the carotid body. Both nonsense and missense mutations *SDHD* were identified in eight unrelated families with this disorder (4). In spite of the imprinting in the chromosomal region containing *SDHD*, the gene appears *not* to be imprinted. In fact, the CybS defect in tumors is not due to parental-allele-specific transcription, but rather to loss of heterozygosity of the normal maternal allele. Reasons for the limited range of organ involvement that occurs in this syndrome remain unclear, but may be due to monoallelic expression of *SDHD* in the carotid body, or to a specific vulnerability of the carotid body via hypoxic stimulation, providing a selective advantage for tumor cells (8).

Complex IV disorders

Complex IV, or cytochrome *c* oxidase (COX), transfers electrons from cytochrome *c* to molecular oxygen and pumps protons across the inner mitochondrial membrane (22). It is comprised of thirteen subunits: the 3 largest are encoded by mtDNA and the other 10 by nDNA. The mtDNA-encoded subunits have two heme-containing cytochrome prosthetic groups (cytochromes *a* and a_3) (3), as well as three copper atoms (located in the Cu_A and Cu_B sites). Although isolated COX deficiency due to mutations in mtDNA-encoded genes has been associated with myopathies (16) and multisystemic disease (20), no pathogenic mutations in the nuclear-encoded subunits of COX have been found (1, 14). However, three assembly proteins required for the proper function and assembly of COX — SURF1 (45, 55), SCO2 (26), and COX10 (49) — have now been associated with encephalomyopathies and COX deficiency.

SURF1, a homologue of yeast Shy1p, is a COX assembly protein of unknown function that is imported into mitochondria (47, 53). It is required for the maintenance of COX activity and is possibly involved in the early stages of COX assembly (54). Numerous groups have now confirmed that mutations in *SURF1* are associated with COX-deficient Leigh syndrome (9, 28, 42, 44-46, 54, 55), although the prevalence within different populations seems to vary. Patients usually present in early infancy with failure to thrive, and with brainstem and respiratory abnormalities, and die in early to late childhood. Patients have lactic acidosis and typically have lesions in the basal ganglia. Biochemical studies show isolated COX deficiency in muscle and cultured fibroblasts. Histochemistry of muscle biopsies shows reduced COX activity but no ragged-red fibers.

Mutations in *SURF1* are usually frameshifts; the most common mutation is a deletion of 10 bp plus an insertion of an AT dinucleotide at encoded amino acid position 104 in Exon 4 (312del10/insAT) (42, 46). Affected individuals may be homozygotes or compound heterozygotes. Western blot analysis has shown that pathogenic mutations are associated with a loss of protein, due to mRNA instability or rapid protein degradation, or both (47, 53). To date, 25 mutations in *SURF1* have been described.

SCO2 is a mitochondrially-targeted protein thought to be required for the insertion of copper into the mtDNA-encoded subunits I and II of COX (26). Mutations in *SCO2* are associated with hypertrophic cardiomyopathy and encephalopathy that present soon after birth. Affected infants have respiratory difficulties and metabolic acidosis, and die within the first year of life. Biochemical studies in affected tissues (brain, muscle, and heart) showed severe decreases in COX activity, but COX deficiency was less in cultured skin fibroblasts (15, 26). Comparative biochemical and histochemical studies showed that COX deficiency in muscle in patients with mutations in *SCO2* was more severe than in those with mutations in *SURF1* (42). In patients with *SCO2* mutations, neuropathological findings were variable, including heterotopia, gliosis, early capillary proliferation, and atrophy. In contrast to patients with mutations in *SURF1*, no child with *SCO2* mutations had neuropathological findings consistent with Leigh syndrome, possibly because they died before they could manifest these features.

To date, five mutations in *SCO2* have been reported — one nonsense mutation (Q53X) and four missense mutations (E140K, L151P, R171W, and S225F) — in seven unrelated families. Interestingly, all patients to date have been compound heterozygotes, and even more remarkably, the E140K mutation was present in all affected individuals (15, 26, and our unpublished data). All heterozygous carriers were asymptomatic.

COX10 encodes a heme A:farnesyltransferase, which catalyzes the conversion of protoheme to heme O, the immediate precursor of heme A, which is the prosthetic

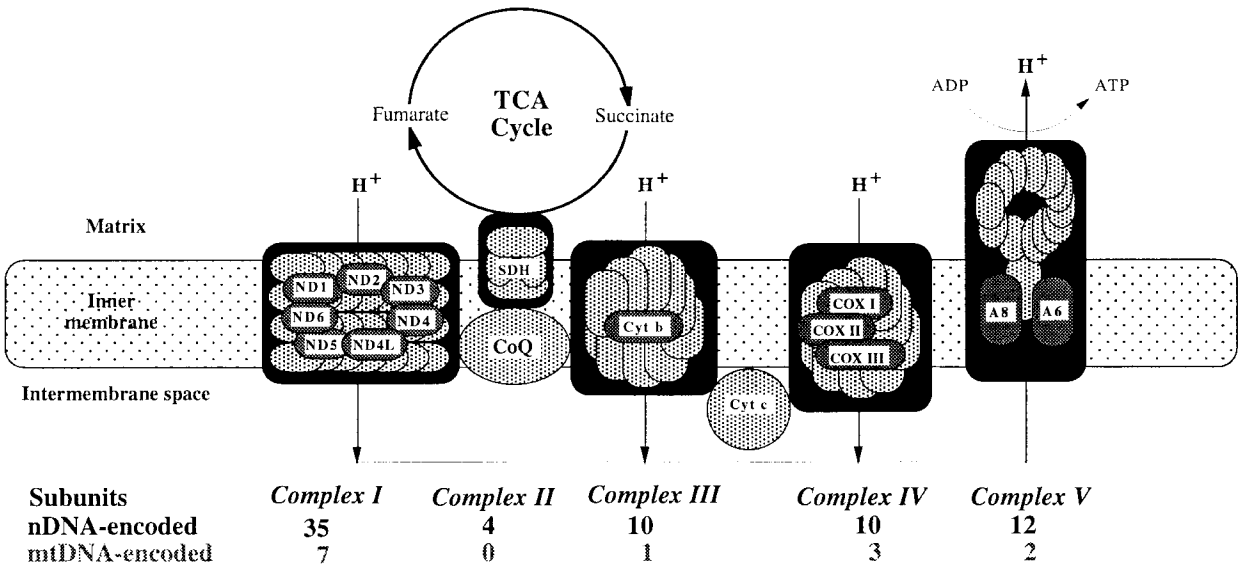

Figure 1. The mitochondrial respiratory chain, showing nDNA-encoded (light-shaded) and mtDNA-encoded (dark-shaded) subunits. Protons (H^+) are first pumped from the matrix to the intermembrane space through Complexes I, III, and IV. They are then pumped back into the matrix through Complex V to produce ATP. Coenzyme Q (CoQ) and cytochrome *c* (Cyt c) are electron transfer carriers.

group of the COX I subunit. Homozygous mutations in exon 4 of *COX10*, converting an asparagine to lysine at amino acid position 204 (N204K), were found in three of nine siblings born to consanguinous parents (49). Both parents and some unaffected siblings were heterozygotes for this mutation. Neurological features included hypotonia, myopathy, ataxia, and seizures. Lactic acidosis and renal proximal tubulopathy were also present in one child. Biochemical studies showed reduced COX activity in muscle, lymphocytes, and fibroblast cell lines. Western blot analysis showed that this mutation was associated with almost complete lack of COX II, moderately reduced levels of COX III and VIc, and mild reductions in the other COX subunits. Complementation studies using yeast *COX10* null mutants showed that, compared to the wild-type human protein, the mutant human protein had markedly reduced function (49).

Complex V

Complex V (ATP synthase or F_1F_0 ATPase) synthesizes ATP from ADP using the proton gradient generated by the respiratory chain (see Figure 1). It is comprised of 2 mtDNA-encoded subunits and 12 subunits encoded by nDNA. While mutations in the mitochondrial-encoded components have been associated with human disease (see article by DiMauro and Andreu in this issue), no pathogenic mutations involving the nDNA-encoded subunits have yet been found.

One candidate disorder, however, is Luft disease, which might be due to defects in Complex V. First described in 1962 (19), Luft disease is a rare condition that presents in adolescence with fever, heat intolerance, profuse sweating, polyphagia, polydipsia, tachycardia, and mild to moderate weakness (10). Basal metabolic rate is elevated but patients are euthyroid. Muscle biopsies from the two known patients had RRFs and capillary proliferation, while polographic studies on isolated muscle mitochondria showed loose coupling of proton flow to ATP synthesis. Defective calcium handling by mitochondria, with abnormal spontaneous release, has also been documented (10). Notably, fibroblast mitochondria from a patient with Luft disease seem to lack the Pullman-Monroy inhibitor, a mitochondrial ATPase inhibitor protein, in the face of normal ATPase, ATP-synthetase, and SDH activities (52). However, the molecular basis for this rare disorder remains elusive.

Coenzyme Q10

Coenzyme Q_{10} (CoQ_{10}) is a lipophilic quinone that accepts electrons from Complex I and Complex II and transfers them to Complex III (Figure 1). Partial defects (20-30%) of CoQ_{10} have been reported in association with KSS and a number of undefined myopathies (12, 21, 24, 25, 56), but CoQ_{10} concentration in muscle was extremely low in four patients (5, 38, 41). These seem to

form a homogeneous subgroup, typically presenting in early to late childhood with exercise intolerance, weakness, myoglobinuria, and cerebral dysfunction. Muscle weakness is generally mild, and myoglobinuria may be induced by exercise (38), fever, or seizures (41). Ptosis and external ophthalmoplegia have also been reported (38). Cerebral dysfunction includes seizures (generalized or complex partial), cognitive impairment, and cerebellar ataxia. Cardiac involvement, if present, is mild (38). Serum creatine kinase levels may be mildly to moderately elevated between attacks of myoglobinuria, and serum lactate levels are increased. Biochemical assays of Complex I+II and Complex I+III activities (all of which require CoQ_{10}) show low activities. Muscle biopsies from patients show RRFs and excess lipid droplets, and CoQ_{10} levels ranged from 3-25% of normal control values. CoQ_{10} levels were normal in serum, fibroblasts, and lymphoblast cell lines of affected patients, implying that this is a tissue specific disorder. To date, no known mutations responsible for defective CoQ_{10} activity have been identified. However, identification of patients is extremely important, as symptoms may improve dramatically with CoQ_{10} administration (5).

Discussion

Although the first nuclear-encoded gene defect of the respiratory chain was reported six years ago, the past two years have seen the identification of many more such errors. These mutations are not only in proteins that comprise the enzyme complexes themselves, but are also in "ancillary" proteins required for their assembly and proper functioning. With the sequencing of the entire human genome, the development of DNA chip arrays, and a greater understanding of the role that the respiratory chain plays in the pathophysiology of human disease, it is predictable that many more nuclear mutations will be identified in the near future.

In contrast to the diversity of phenotypic expression associated with mitochondrial DNA mutations, each mendelian-inherited mitochondrial disorder usually causes a distinct clinical phenotype, such as Leigh syndrome or, less commonly, fatal infantile cardioencephalomyopathy. Also, while disorders due to mtDNA mutations often have late onset, pathogenic mutations in nuclear genes controlling respiratory chain complexes seem to cause diseases of infancy or early childhood. This may be because recessive mendelian disorders tend to be "all-or-none" phenotypically, in the sense that both alleles must be mutated for the disease to be expressed. In contrast, most mtDNA-based disorders are heteroplasmic (albeit at high mutational loads), implying that homoplasmic levels of those mutations would be as severe, if not more so, than "homoplasmic" (i.e. homozygous, compound heterozygous, or hemizygous) nDNA-encoded mutations.

Perplexingly, pathogenic mutations have been identified only in nuclear-encoded polypeptides of Complexes I and II, but not (at least, not yet) in those of Complexes III, IV, or V. This may be due to the fact both Complexes I and II feed into ubiquinone "in parallel," and the organism may be able to cope with the loss of either complex separately — something that cannot be said for complexes III or IV, which are downstream of ubiquinone and are in "series" in the respiratory chain. In fact, in some organisms (most notably, the yeast *Saccharomyces cerevisiae*) Complex I is not even present! Similarly, Complex V is absolutely required for oxidative ATP synthesis and thus, cannot be bypassed.

It is worth noting that the mtDNA-encoded subunits of complexes I and IV specify the essential catalytic activities of the holoprotein (witness the absence of "nDNA-encoded" subunits in these complexes in prokaryotes). While the biological importance of the mtDNA-encoded "core" subunits is obvious, the relative contribution of the nDNA-encoded subunits to overall function (e.g. modulation of activity commensurate with varying metabolic needs of the cell; tissue-specific activity) is far less clear. As is the case with complex V, however, it is possible that the functions of the nDNA-encoded subunits of Complexes III and IV are as essential as those of the mtDNA-encoded subunits, in which case mutations in nuclear genes would lead to embryonic lethals. It is noteworthy in this regard that even the known assembly mutations in Complex IV in infants who at least survive birth do not obliterate COX enzyme activity completely, but rather allow affected tissues to maintain a residual (albeit low, and ultimately insufficient) level of normal respiratory function. Of course, mutations in "less critical" proteins within the respiratory chain complexes may result in less deleterious effects or in subclinical disease, and may thus go undetected.

Finally, mutations in Complex II in hereditary paraganglioma represent the first errors in a respiratory chain gene associated causally with neoplastic transformation (4). While mutations in mitochondrial DNA have been postulated to play a role in tumorigenesis (39), no solid evidence supporting this concept had been obtained prior to this finding. It is possible that abnormal respiratory chain function may play an important role in the activation or inhibition of tumor suppressor

genes, but identification of other mutations and their pathophysiologic mechanisms will be necessary to confirm this notion.

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