INVITED EDITORIAL
Getting to the Nucleus of Mitochondrial Disorders: Identification of Respiratory Chain–Enzyme Genes Causing Leigh Syndrome

Hans-Henrik M. Dahl

The Murdoch Institute, Royal Children's Hospital, Melbourne

In 1951, Denis Leigh first described a striking neuropathology that occured in a child who died of a neurodegenerative disease (Leigh 1951). The paper focused on the patient's distinct neuropathological abnormalities, which we now associate with Leigh disease, or Leigh syndrome (MIM 256000). Definite diagnosis of this neurodegenerative disorder still depends on the identification, either in postmortem samples or by use of neuroimaging techniques, of the characteristic neuropathology of focal, bilaterally symmetrical spongiform lesions, especially in the thalamus and brain stem regions. This neuropathology is associated with demyelination, vascular proliferation, and gliosis. Onset is usually within the 1st year of life but can be later, and the clinical features and course of the disease vary among patients. Symptoms often include motor and/or intellectual retardation, abnormal breathing rhythm, nystagmus, opthalmoparesis, optic atrophy, ataxia, and dystonia. Death is usually within 2 years of onset, although slower progression is not unusual.

It was originally suggested that the biochemical defect in Leigh syndrome is a block in thiamine metabolism, but it is now clear that the defect is a result of severe deficiency in mitochondrial ATP production. Leigh syndrome is one of the most commonly recognized disorders of mitochondrial energy production and can be regarded as one part of the spectrum of these disorders. For example, some mutations in mtDNA, when present in high proportions in affected tissues, will cause Leigh syndrome, whereas lower levels of mutant mtDNA will cause milder phenotypes. In a similar manner, severe mutations in some nuclear genes appear to result in Leigh syndrome, but functionally less-severe mutations in the same genes give rise to different clinical presentations. Because of this and because of the difficulty of establishing a definite clinical diagnosis, it could be argued that, for many patients, a more appropriate term for their condition would be "Leigh-like syndrome" or "mitochondrial encephalopathy."

Although initial observations suggested autosomal recessive inheritance, it soon became clear that inheritance could also be autosomal dominant, X-linked, or maternal, which indicated that causative genes exist in both the nuclear and the mitochondrial genome. Genetic heterogeneity has been confirmed by identification of functional or molecular defects in several enzyme systems involved in mitochondrial energy production, including the pyruvate dehydrogenase complex (PDHC), respiratory chain complexes I, II, and IV (cytochrome c oxidase [COX]), and the mitochondrial-encoded ATPase6 subunit (DiMauro and De Vivo 1996). Because of difficulties associated with the measurement of the activity of these enzyme complexes, especially complex I, there is some variation in the estimate of the relative incidence of the various causes. We published a study of 67 patients with Leigh or Leigh-like syndrome (Rahman et al. 1996) and were able to determine the cause in approximately two-thirds of the cases (table 1). These frequencies generally agree with those reported by Morris et al. (1996).

A number of mutations causing Leigh syndrome have been identified in mtDNA. Analysis of these mutations illustrates the complex genotype-phenotype correlation in mtDNA disorders. The T→G and T→C mutations at nucleotide 8993 in the ATPase6 gene are the most common. The T8993G mutation initially was associated with neuropathy, ataxia, and retinitis pigmentosa but later was found in patients with Leigh syndrome (Tatuch et al. 1992). As with other mtDNA mutations, the clinical presentations can vary significantly among affected individuals, most likely as a result of variations in the proportions of mutant DNA (heteroplasmy) in the different tissues. One would expect the Leigh syndrome patients to have a high proportion of T8993G mutations in affected tissues. Leigh syndrome patients who carry the A→G mutation at nucleotide 8344 in the $tRNA^{Lys}$ gene (a mutation that is ordinarily associated with

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Address for correspondence and reprints: Dr. Hans-Henrik M. Dahl, The Murdoch Institute Royal Children's Hospital, Parkville, Melbourne, Victoria 3052, Australia. E-mail: dahl@cryptic.rch.unimelb.edu.au

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Table 1

Causes of Leigh or of Leigh-like Syndrome

Defect	Frequency[a] (%)	Component(s) of Affected Genes
mtDNA	18	ATPase6 (nt 8993, nt 9176), $tRNA^{Lys}$ (nt 8344), mtDNA deletions
PDHC	10	E1α subunit
Complex I	19	NDUFS8
COX	14	SURF-1
Other	39	Flavoprotein subunit of complex II mtDNA depletion?

[a] Rahman et al. 1996.

MERRF [myoclonic epilepsy with ragged red fibers] syndrome), the T→C mutation at nucleotide 9176 in the ATPase6 gene, or mtDNA deletions are also presumed to carry high mutant loads in specific tissues. mtDNA depletion, which is likely to be caused by a nuclear-gene defect, has also been associated with Leigh syndrome (Morris et al. 1996).

Cloning of the subunits of the PDHC enabled us to search for mutations in patients with Leigh syndrome resulting from deficiency of PDHC activity. In the majority of these patients, the defect occurs in the catalytic subunit, E1α. The X linkage of the *PDHC E1*α gene partially explains the predominance of males with this disorder. However, females with Leigh syndrome and a mutation in PDHC E1α have also been identified. Whether a male develops Leigh syndrome will largely depend on the functional severity of the E1α mutation. In females, the clinical presentation will also depend on the X chromosome–inactivation pattern in different tissues, especially in the brain (Dahl 1995). A mutation has also been found in the flavoprotein subunit of complex II in two siblings with Leigh syndrome (Bourgeron et al. 1995).

Mutations in mtDNA or in PDHC subunits appear to account for approximately one-third of Leigh syndrome patients. If, however, analysis of defects in these genes has been relatively straightforward and productive, then the molecular analysis of the other common causes of Leigh syndrome has been slow and frustrating. This issue of the Journal includes two articles that identify mutations in nuclear genes leading to Leigh syndrome. Tiranti et al. (1998 [in this issue]) have identified defects in the Surfeit-1 (SURF-1) protein that result in COX deficiency, and Loeffen et al. (1998 [in this issue]) have found mutations in the NADH:ubiquinone oxidoreductase 23-kD subunit (NDUFS8) protein that cause complex I deficiency. Two different approaches were used to find the causative genes.

The success of a direct approach is demonstrated by Loeffen et al. (1998). An increasing number of nuclear genes encoding complex I subunits are being characterized, and a logical response is to search for mutations in these genes. Often, bacteria, yeast, or bovine complex I subunit genes are used to identify the human homologues. When Loeffen et al. analyzed patients with complex I deficiency for mutations in the *NDUFS8* gene, they found that one of their patients, a child who died from Leigh syndrome, had amino acid substitutions in both alleles. This does not definitively prove that these changes are the causative mutations and that the *NDUFS8* gene is the defective gene, but the type and location of the amino acid substitution supports the authors' conclusions. NDUFS8 is a highly conserved and important structural complex I subunit (Chevallet et al. 1997). It is, therefore, conceivable that changes in NDUFS8 can severely affect complex I function and cause Leigh syndrome.

In their search for candidate genes, Loeffen et al. (1998) took advantage of data generated by analysis of respiratory chain–enzyme defects in other organisms, especially in yeast. Such information also proved very useful for Tiranti et al. (1998). Better comprehension of complex I deficiency and of Leigh syndrome may have to wait until most or all of the >35 nuclear-encoded subunits have been characterized. Even then, it is unlikely that all disease-causing mutations will be in the genes for these structural subunits. The study of COX deficiency in Leigh syndrome ($LD^{[COX-]}$) suggests that the picture is more complex than anticipated. COX consists of 10 nuclear-encoded and 3 mtDNA-encoded structural subunits. Two tissue-specific isoforms are found for 2 of the nuclear-encoded subunits. The nucleotide sequences of all 15 genes are known. Pedigree analysis (Van Coster et al. 1991) and cybrid (Tiranti et al. 1995) and complementation studies (Munaro et al. 1997) have so far shown that $LD^{(COX-)}$ is inherited as an autosomal recessive condition. The 12 nuclear-encoded COX genes are obvious candidate genes for $LD^{(COX-)}$. Extensive investigations in several laboratories, however, have failed to find defects in any of these genes (Adams et al. 1997; H.-H. M. Dahl, D. R. Thorburn, and L. Peters, unpublished data; M. Hirano, personal communication). This failure would indicate that the defects are in genes that are involved in the processing, modification, mitochondrial import, or assembly of the COX complex. This perhaps is not surprising, since genetic analysis of yeast mutants has revealed that an unexpectedly large number of genes (>36) are required for generation of functional COX (McEwen et al. 1986; Tzagoloff and Dieckmann 1990).

How can we map and identify the human genes causing mitochondrial dysfunction, such as $LD^{(COX-)}$, when analysis of the obvious candidate genes is unsuccessful? Tiranti et al. (1998) show that a locus for $LD^{(COX-)}$ is located on chromosome 9q34-qter. Identification of this locus has been achieved by use of a novel and ingenious approach (fig. 1). mtDNA was eliminated from a panel

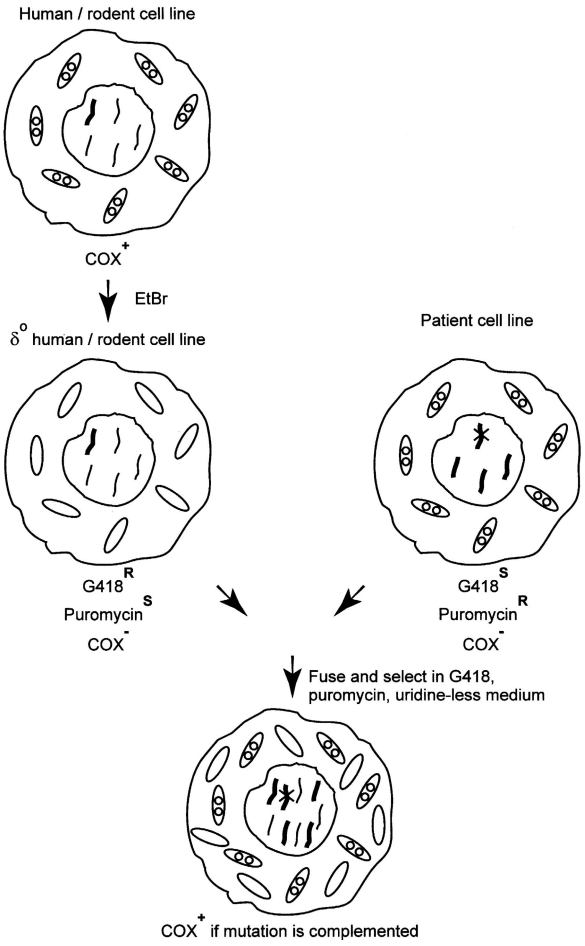

Figure 1 Schematic outline of complementation and selection strategy (Tiranti et al. 1998). Human chromosomes are shown as thick lines, mouse chromosomes and are shown as thin lines. Mitochondria and mtDNA are also indicated. The fused cell line will be COX positive if the mutation is complemented by the wild-type gene on the human chromosome(s) supplied by the human/rodent cell line.

of rodent/human cell hybrids expressing a selectable marker (e.g., G418 resistance) and containing one or more human chromosomes. These were fused with a patient cell line that had been made resistant to puromycin. Fused cells were isolated in medium without uridine but containing G418 and puromycin and were analyzed by use of COX immunohistochemistry and enzyme analyses. When the rodent/human cell supplied a complementing human chromosome (in this case chromosome 9), the fused cells became COX positive. The success of this approach is dependent on the rodent background not interfering with the COX phenotype. This was shown to be the case in the $LD^{(COX-)}$ complementation group studied, and it could be a common feature in other mitochondrial disorders caused by mutations in nuclear genes.

Munaro et al. (1997) previously had demonstrated that all their $LD^{(COX-)}$ patients belonged to one complementation group. This allowed them to perform linkage analysis of 10 families with chromosome 9 markers and to map the disease locus to a 9-cM region between markers D9S1847–D9S1826. This region does not code for any COX subunit genes, but analysis of candidate genes showed that the probands in all 10 $LD^{(COX-)}$ families had mutations in the gene coding for SURF-1. SURF-1 is a mitochondrial protein of unknown function, which affects respiratory chain and, especially, COX function. It is surprising that, although the $LD^{(COX-)}$ patients belong to one complementation group, there is no suggestion of a founder mutation. Instead, nine different mutations were characterized. Further studies have shown that not all $LD^{(COX-)}$ patients belong to the same complementation group (Brown and Brown 1996; Tiranti et al. 1998; D. R. Thorburn and H.-H. M. Dahl, unpublished data). These results indicate that $LD^{(COX-)}$ is genetically heterogeneous and that more mutations and chromosomal loci will be identified. Tiranti et al. have devised a very powerful way of mapping other nuclear genes involved in mitochondrial dysfunction, not just those that cause $LD^{(COX-)}$.

There are several reasons why the articles by Tiranti et al. and Loeffen et al. are exciting. They outline ways to identify nuclear-encoded mitochondrial genes that will provide new information on the pathways that lead to successful import, assembly, and function of respiratory chain enzymes. Furthermore, the genes for $LD^{(COX-)}$ are likely to belong to a group of nuclear genes controlling mitochondrial function, similar to the recently identified genes for Friedreich ataxia and spastic paraplegia (Campuzano et al. 1996; Casari et al. 1998). The use of these elegant approaches is likely to be a rewarding exercise. These approaches eventually will increase our understanding of the complex interactions between the mitochondrial and nuclear genomes and will shed light on the molecular causes of this group of relatively common but genetically and clinically complex and poorly understood neurodegenerative disorders.

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Leigh syndrome [MIM 256000])

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