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Cloning of the human NADH: ubiquinone oxidoreductase subunit B13: localization to Chromosome 7q32 and identification of a pseudogene on 11p15

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Mitochondrial dysfunction is becoming increasingly recognized as a cause of cardiovascular and neuromuscular diseases. Defects of the mitochondrial respiratory chain represent a heterogeneous group of disorders that range in presentation from severe multisystem organ failure in the neonatal period to exaggerated fatigue with exercise (Shoffner and Wallace 1995). To date, many of the described mitochondrial defects responsible for human diseases have involved the mitochondrial NADH:ubiquinoneoxidoreductase enzyme (Complex I), which catalyzes the transfer of electrons from NADH to ubiquinone, couples with ADP phosphorylation (Schapira et al. 1988). This complex is composed of at least 40 proteins, seven of which are encoded by mitochondrial DNA and the remainder by nuclear genes (Walker et al. 1992). Complex I defects have been noted in patients with a variety of neuromuscular disorders, including patients with Parkinson's disease (Parker et al. 1989). Recently, one component of this complex, the B22 subunit, was proposed as a candidate gene for the Branchio-oto-renal syndrome, an autosomal dominant disorder characterized by hearing loss, renal abnormalities, and cervical fistulae (Gu et al. 1996). Some patients with severe Complex I deficiency have been demonstrated to have reduced amounts of the nuclear-encoded B13 subunit as well as a marked diminution of the 24-kDa subunit (Moreadith et al. 1987; Morgan-Hughes et al. 1988). Although the function of the B13 subunit is unknown, it demonstrates significant homology to a fungal protein, the 29.9-kDa subunit from the Complex I of *Neurospora crassa* (Walker et al. 1992). The evolutionary conservation of this protein and its reduced expression in patients with severe Complex I deficiency suggest that it may have functional domains that are important for the understanding of Complex I disorders.

During the course of a physical mapping project on Chromosome (Chr) 11p15.5, we identified sequence from the end of cosmid clone cCI-253 (gift from Yusuke Nakamura) with a high degree of similarity to the bovine NADH:ubiquinone oxidoreductase subunit B13. From the genomic sequence, we constructed a sequence tag site (STS) corresponding to the putative carboxyl and 3′ untranslated sequence, using the bovine gene as a reference. These STSs were amplified from Chr 11 cosmids (Chr 11-specific cosmid library, LA11NC01, from L. Deaven, Los Alamos National Laboratory) that spanned the region. the PCR product was radiolabeled and used to screen an arrayed infant brain cDNA library (Adams et al. 1993). Four cDNA clones of varied sizes were isolated, all with identical sequences in the overlapping intervals. Two apparently identical 1.7-kb clones contained a Kozak consensus start sequence (Kozak 1987). A single clone was sequenced (Genbank accession: U64028) on both strands with fluorescent cycle sequencing (fmol cycle sequencing kit, Promega Inc., Madison, Wis.). This clone contains a single 348-nucleotide open reading frame (116 amino acids) and a relatively large 3′ untranslated region (1,092 nucleotides). During the course of this work, Pata and colleagues have deposited a similar sequence into Genbank (accession number: U53468). Except for some differences in the 3′ untranslated region, our sequence is identical to that deposited by Pata and associates. It is unknown whether the differences between the sequence reported here and that from Pata and colleagues are naturally occurring variants or sequencing errors.

The nucleotide sequence of this clone was 88% and 83% identical over the predicted open reading frame compared with bovine (accession number: X63218) and rat B13 (accession number: D86215) subunit genes respectively. The position of the initiation and termination codons was conserved. Sequence immediately following the translation start site encodes a series of positively charged amino acids, which are necessary for the transit of the protein into the mitochondrion (Neupert et al. 1990). The predicted human B13 polypeptide sequence is 88% and 83% identical compared with the bovine and rat proteins (Fig. 1). Interestingly, the human protein differs from the rat and bovine protein at ten residues where the rat and cow also differ from each other. These regions of difference may indicate residues not evolutionarily constrained by function.

To determine the chromosomal localization of the B13 subunit gene, we designed an STS from the 3′ untranslated sequence of the cDNA where the cDNA significantly diverges from the Chr 11 sequence. A monochromosome somatic cell hybrid (SCH) panel was screened with this STS, and only the SCH containing human Chr 7 was positive (data not shown). This STS was then used to isolate a P1 clone (DMPC-HFF #1, clone 56A9; Shepard et al. 1994) with a PCR-based strategy. Fluorescent in situ hybridization with this P1 localized the B13 gene to Chr 7q32 (Fig. 2). Extensive sequencing of the genomic DNA on 11p15.5 revealed that there was high similarity to the true gene, but that there was a one base pair deletion in the ''coding sequence'' of the pseudogene and it did not contain a Kozak consensus start sequence. This similarity was observed in both the coding and 3′ untranslated sequence, but the nucleotide differences were not concentrated at the third or ''wobble'' base of the coding sequence. Furthermore, there was no evidence of a splice acceptor site at the 5′ extent of the pseudogene, indicating that it was not likely to be an exon from a larger transcript that had incorporated a B13 functional domain. The sequence was searched against the Genbank and dbEST databases with the BLAST program (Altschul et al. 1990). In addition to

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Note: The nucleotide sequence data reported in this paper have been submitted to Genbank and have been assigned the accession number U53468.

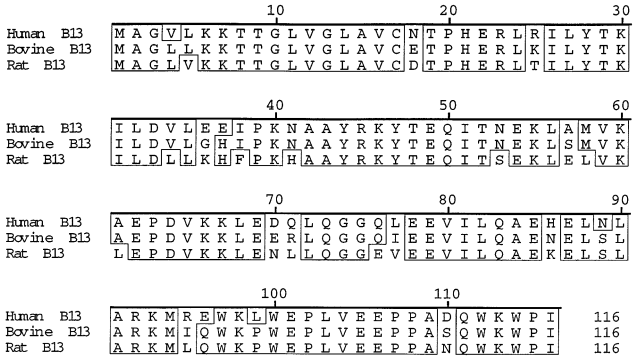

Fig. 1. Conserved protein sequence between the human, bovine, and rat B13 genes. Protein sequences were aligned with the Clustal algorithm (DNAStar program); identical residues are boxed. The bovine and rat B13 sequences were retrieved from Genbank, accession numbers X63218 and D86215 respectively. DNA and protein sequence reported here have been given the Genbank accession number U53468.

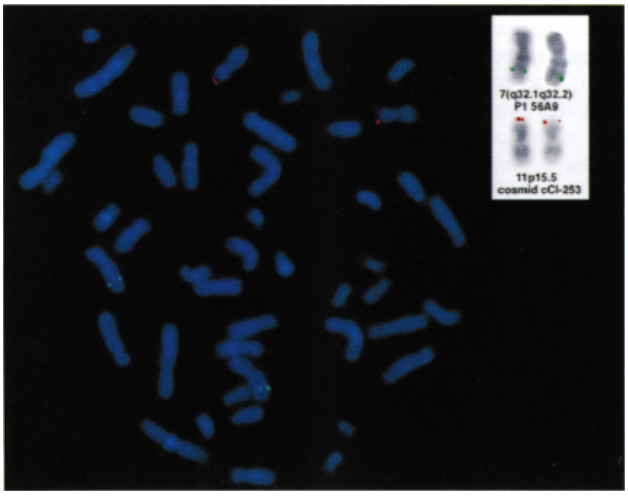

Fig. 2. Two-color metaphase fluorescent in situ hybridization analysis with a P1 (56A9) containing the B13 gene (green fluorescence, fluorescein isothiocyanate, FITC) and a cosmid (cCL-253) containing the pseudogene (in red fluorescence, tetramethylrhodamine isothiocyanate, TRITC) as probes. Chromosomes were counterstained with DAPI. **Inset:** Inverse DAPI image showing chromosome banding and sublocalization of the probes. Staining and image acquisition were performed as described in Schröck and coworkers (1996).

strong similarity to the bovine and rat B13 subunit genes, several human ESTs were identified. These were derived from prostate, infant brain, atrial and skeletal muscle cDNA libraries demonstrating widespread expression of this gene. Close examination of the sequence from all of these ESTs revealed them to be identical to the 3′ end of the Chr 7-derived B13 gene. Furthermore, screening of the infant brain cDNA library with probes from the Chr 11p15.5 locus yielded only cDNAs corresponding to the Chr 7 sequence. Therefore, there is no evidence that the Chr 11p15.5 locus encodes a functional protein, and it probably represents a processed pseudogene of the B13 gene. One other pseudogene of the human B13 gene has been described near the ribosomal protein S6 (RPS6) gene on Chr 9 (Pata and Metspalu 1993).

The cloning of the human B13 subunit of the mitochondrial NADH:ubiquinone oxidoreductase may aid the understanding of Complex I disorders. The potential role of this gene in human disease processes that have been attributed to Complex I deficiencies can now be examined. The extreme conservation of the B13 protein sequence between three different mammals will aid in this analysis.

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References

Adams, M.D., Soares, M.B., Kerlavage, A.R., Fields, C., Venter, J.C. (1993). Rapid cDNA sequencing (expressed sequence tags) from a directionally cloned human infant brain cDNA library. Nature Genet., 4, 373–80.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. 215, 403–10.

Gu, J.Z., Lin, X., Wells, D.E. (1996). The human B22 subunit of the NADH-ubiquinone oxidoreductase maps to the region of chromosome 8 involved in branchio-oto-renal syndrome. Genomics 35, 6–10.

Kozak, M. (1987). An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res. 15, 8125–8148.

Moreadith, R.W., Cleeter, M.W.J., Ragan, I.C., Batshaw, M.L., Lehninger, A.L. (1987). Congenital deficiency of two polypeptide subunits of the iron-protein fragment of mitochondrial complex I. J. Clin. Invest. 79, 463–467.

Morgan-Hughes, J.A., Schapira, A.H.V., Cooper, J.M., Clark, J.B. (1988). Molecular defects of NADH-ubiquinone oxidoreductase (Complex I) in mitochodrial diseases. J. Bioenerg. Biomembr. 20, 365–382.

Neupert, W., Hartl, F.U., Craig, E.A., Pfanner, N. (1990). How do polypeptides cross the mitochondrial membranes? Cell 63, 447–50.

Parker, W.D. Jr., Boyson, S.J., Parks, J.K. (1989). Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann. Neurol. 26, 719–723.

Pata, I., Metspalu, A. (1993). A dinucleotide repeat polymorphism at the ribosomal protein S6 (RP6) gene. Hum. Mol. Genet. 2, 1749.

Schapira, A.H.V., Cooper, J.M., Morgan-Hughes, J.A., Patel, S.D., Cleeter, M.J.W., Ragan, C.I., Clark, J.B. (1988). Molecular basis of mitochondrial myopathies: polypeptide analysis in complex-I deficiency. Lancet 8584, 500–503.

Schröck, E., Badger, P., Larson, D., Erdos, M., Wynshaw-Boris, A., Reid, T., Brody, L. (1996). The murine homolog of the human breast and ovarian cancer susceptibility gene *Brcal* maps to mouse chromosome 11D. Hum. Genet. 97, 256–259.

Shepard, N.S., Pfrogner, B.D., Coulby, J.N., Ackerman, S.L., Vaidyanathan, G., Sauer, R.H., Balkenhol, T.C., Sternberg, N. (1994). Preparation and screening of an arrayed human genomic library generated with the P1 cloning system. Proc. Natl. Acad. Sci. USA 91, 2629–2633.

Shoffner, J.M., Wallace, D.C. (1995). Oxidative phosphorylation diseases. In The Metabolic and Molecular Bases of Inherited Disease, C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle, eds. (New York: McGraw-Hill, Inc.) pp. 1535–1609.

Walker, J.E., Arizmendi, J.M., Dupuis, A., Fearnley, I.M., Finel, M., Medd S.M., Pilkington, S.J., Runswick, M.J., Skehel, J.M. (1992). Sequences of 20 subunits of NADH:uniquinone oxidoreductase from bovine heart mitochodria. J. Mol. Biol. 226, 1051–1072.