Mitochondrial Neurogastrointestinal Encephalopathy Due to Mutations in RRM2B

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Background: Mitochondrial neurogastrointestinal encephalopathy (MNGIE) is a progressive neurodegenerative disorder associated with thymidine phosphorylase deficiency resulting in high levels of plasma thymidine and a characteristic clinical phenotype.

Objective: To investigate the molecular basis of MNGIE in a patient with a normal plasma thymidine level.

Design: Clinical, neurophysiological, and histopathological examinations as well as molecular and genetic analyses.

Setting: Nerve and muscle center and genetic clinic.

Patient: A 42-year-old woman with clinical findings strongly suggestive for MNGIE.

Main Outcome Measures: Clinical description of the disease and its novel genetic cause.

Results: Identification of mitochondrial DNA depletion in muscle samples (approximately 12% of the control mean content) prompted us to look for other causes of our patient's condition. Sequencing of genes associated with mitochondrial DNA depletion—POLG, PEO1, ANTI, SUCLG1, and SUCLA2—did not reveal deleterious mutations. Results of sequencing and array comparative genomic hybridization of the mitochondrial DNA for point mutations and deletions in blood and muscle were negative. Sequencing of RRM2B, a gene encoding cytosolic p53-inducible ribonucleoside reductase small subunit (RIR2B), revealed 2 pathogenic mutations, c.329G>A (p.R110H) and c.362G>A (p.R121H). These mutations are predicted to affect the docking interface of the RIR2B homodimer and likely result in impaired enzyme activity.

Conclusions: This study expands the clinical spectrum of impaired RIR2B function, challenges the notion of locus homogeneity of MNGIE, and sheds light on the pathogenesis of conditions involved in the homeostasis of the mitochondrial nucleotide pool. Our findings suggest that patients with MNGIE who have normal thymidine levels should be tested for RRM2B mutations.

Arch Neurol. 2009;66(8):1028-1032
for MNGIE but whose plasma thymidine level was nor-
mal. Further analysis showed severe mtDNA depletion
in muscle tissue, and sequencing analysis revealed 2 patho-
genic mutations in the RRM2B gene.

METHODS

CLINICAL PRESENTATION

A 42-year-old woman of mixed African American and Latin Ameri-
can descent was referred to us for evaluation of ophthalmople-
gia, ptosis, gastrointestinal dysmotility, cachexia, peripheral neu-
ropathy, and brain magnetic resonance imaging changes (Figure 1).
She was in good health until age 30 years, when she
developed recurrent and severe episodes of nausea and vomiting
due to gastrointestinal dysmotility. In the same year, she was hos-
pitalized with systemic bacterial infection and received intrave-
nous gentamicin sulfate, which was followed by the develop-
ment of sensorineural hearing loss. Owing to her gastrointestinal
problems, she lost significant weight; in fact, she weighed 30 kg
(body mass index [calculated as weight in kilograms divided by
height in meters squared], 12.5) a year before her presentation
to us. Since age 37 years, she had progressive restriction of eye
movements, ptosis, micronystagmus, dysarthria, unsteady gait,
generalized muscle weakness, and loss of deep tendon reflexes.
Her sensorium was preserved. Her family history was negative
for similarly affected relatives.

Her initial diagnostic evaluation revealed normal levels of
plasma thymidine (100 mmol/L; reference range, <150
mmol/L), serum aminotransferases, creatine phosphokinase,
and plasma amino acids as well as a normal acylcarnitine
profile. Her plasma lactate level was mildly elevated (27.9 mg/dL
[to convert to millimoles per liter, multiply by 0.111]; refer-
ence range, 1.8-18.0 mg/dL). Cerebrospinal fluid analysis
showed mild elevation of the levels of total protein, IgG,
myelin basic protein, and lactate. Funduscopic evaluation,
echocardiography, and needle electromyography results were
normal. Nerve conduction studies revealed normal sural
potentials, generalized motor slowing (32-37 m/s), and pro-
longed distal latencies consistent with demyelinating neu-
ropathy. Brain magnetic resonance imaging showed increased
T2-weighted signal in the basal ganglia and patchy
T2-weighted signals throughout the periventricular and sub-
cortical white matter (Figure 1). The muscle biopsy revealed
mild variation of fiber size (Figure 2A), increased endo-
mysial connective tissue, rare ragged red fibers on modified
Gomori trichrome stain (Figure 2B), and numerous ragged
blue fibers on succinate dehydrogenase reaction (Figure 2C).
The overall findings were consistent with mitochondrial
myopathy and mild neurogenic atrophy with reinnervation.
The activity of the respiratory chain complexes in muscle was
within the normal range (data not shown).

ANALYSES

Spectrophotometric analysis of the respiratory chain complexes
was performed according to previously described pro-
tocols.10 The coding exons and the immediate flanking intronic
sequences of RRM2B, POLG1, ANT1, PEO1, SUCLG1,
and SUCLA2 were amplified by polymerase chain reaction
and sequenced in the forward and reverse directions using au-
tomated fluorescence dideoxy-sequencing methods. The poten-
tial effects of mutants on the protein structure were estimated
by comparing the local environments of mutants11 among the
homologs of the ribonucleoside reductase small subunit (RIR2B)
protein family. Nuclear DNA and mtDNA copy numbers were
determined by real-time quantitative polymerase chain reaction
according to a previously validated protocol.12
RESULTS

DNA ANALYSIS

Results of mtDNA analysis in peripheral blood and muscle were negative for deleterious point mutations by se-

quencing and deletions by array comparative genomic hybridization. A homoplasmic unclassified missense vari-

ant, m.15077G>A (E111K, CytB), was detected and previously reported in the Polysite database (http://www.genpat.uu.se/mtDB/) with a frequency of 2702:2 (A:G). A homoplasmic variant, m.16017T>C, in transfer RNA located at the first base of the stem region of the amino acid acceptor arm was detected. The clinical conse-

quences of these nucleotide changes are unknown but likely represent benign variants. Sequencing of POLG,

ANT1, PEO1, SUCLG1, and SUCLA2 did not identify del-

erious changes. Sequencing of RRM2B revealed c.329G>A (p.R110H) and c.362G>A (p.R121H) muta-

tions. Analysis of parental DNA confirmed that the iden-

tified mutations were in trans configuration.

mtDNA DEPLETION

The mtDNA content in blood and muscle was measured in duplicates and confirmed by repeat runs. Analysis of the mtDNA content performed on the muscle biopsy specimen showed 12% of the control mean content, but no depletion was detected in peripheral blood.

PREDICTED STRUCTURAL CHANGES ON RIR2B

To evaluate the effect of the mutations R110H and R121H, we examined the evolutionary history of each mutant in its local structural environment composed of the residues within 0.50 nm. The analyzed homologs consisted of 256 sequences coming from 165 different sources (including animals, plants, bacteria, and viruses). The metazoan se-

quences clustered in the phylogenetic tree and consisted of 60 sequences from 32 different species. Substitution R121H occurs in only 2.3% of homologs, which share less than 30% of the total sequence identity and only 8% of the sequence identity among the contacts in position 121. Substitution R110H is seen in 1.2% of homologs sharing less than 70% of the total and 46% of the local sequence iden-

tities. Both positions are almost invariant in RIR2B in metazoan. No homologs simultaneously carried histidine in both positions. Residues R110 and R121 are located in close proximity to each other and to the known homodimer RIR2B docking interface (Figure 3). Together these data are con-

sistent with R121H and R110H being jointly sufficiently deleterious to affect the protein function.

Figure 2. Muscle biopsy suggestive of mitochondrial dysfunction. A, Decreased cytochrome-c oxidase staining (original magnification ×100). B, Modified Gomori trichrome staining showing a ragged red fiber (original magnification ×400). Mild variation in fiber size is seen. C, Succinate dehydrogenase staining showing multiple ragged blue fibers (original magnification ×40). These findings were suggestive of the mitochondrial dysfunction. Other findings included rare denervated fibers and several small fiber-type groups (data not shown).

Figure 3. Consequences of the R110H and R121H mutations on the structure of the ribonucleoside reductase small subunit (RIR2B). R110H and R121H (in red) are within 0.75 nm of each other and straddle the outlined homodimer interface. Each has 13 structural neighbors (light green) that overlap at F111, S112, Q113, and E114 (dark green). The few instances of homologs carrying the R110H substitution have at best only 6 identical structural neighbors (W106, L107, E109, F111, E114, and R186), and the homologs with the R121H substitution have at best just 1 identical structural neighbor (Y124). The figure was generated by using the PyMOL program (DeLano Scientific LLC, Palo Alto, California) and the 2vux Protein Data Bank file (doi:10.2210/pdb2vux/pdb).
CLINICAL OUTCOME

The patient received vitamin C (500 mg 3 times a day), vitamin E (400 units twice a day), coenzyme Q10 (200 mg twice a day), and levocarnitine (1 g 3 times a day). Within 6 months from starting the treatment, she showed normalization of the plasma lactate level and improvement in weight, muscle strength, eye movements, and daily life activities. Her hearing and deep tendon reflexes did not improve.

COMMENT

The RRM2B gene encodes protein RIR2B (p53R2), which is transcriptionally regulated by the tumor suppressor TP53 and plays a key role in the regulation of stress response to various cell-damaging stimuli. Mutations in RRM2B have been reported to cause mtDNA depletion in human subjects and a murine model, demonstrating its essential role in mtDNA replication and repair.

EXPANDING THE CLINICAL SPECTRUM OF RRM2B-RELATED DISORDERS

To our knowledge, a total of 14 patients from 10 nonrelated families and carrying a total of 16 unique mutations in the RRM2B gene have been described to date. The spectrum of clinical findings includes muscle hypotonia, seizures, neonatal-onset vomiting and diarrhea, lactic acidosis, renal tubulopathy, and early childhood mortality. The oldest described patient, aged 36 months, had a more benign clinical course that correlated with the higher mtDNA content, 11% of the control mean content. Skeletal muscle biopsy in the studied patients demonstrated severe mtDNA depletion, ranging between 1% and 11% of the control mean content. The activities of respiratory chain enzymes containing mtDNA-encoded subunits were markedly reduced in most but not all of the patients with RRM2B mutations. Our patient is the oldest described patient with RRM2B mutations with the highest mtDNA content (12% of the control mean content). She initially presented with gastrointestinal dysmotility at age 30 years. We hypothesize that the severe vomiting and diarrhea observed in infants with RRM2B mutations reflect a fundamental gastrointestinal dysfunction and mirror the gastrointestinal dysmotility seen in older patients with MNGIE. Although our patient developed sensorineural hearing loss after gentamicin treatment, results of her whole mitochondrial genome sequencing were negative for m.1555A>G, m.961delT+(C)+, and m.7443A>G mutations associated with mitochondrial nonsyndromic hearing loss and deafness. This raises the possibility that patients with RIR2B dysfunction could be susceptible to aminoglycoside-induced deafness.

EXPANDING THE MOLECULAR MECHANISMS OF MNGIE

Nishino et al. established the causal link between the dysfunction of thymidine phosphorylase and MNGIE. The clinical presentation in our patient closely resembled MNGIE but there were subtle differences. The nerve conduction velocities were decreased but not to the degree typically seen in patients with MNGIE, and brain magnetic resonance imaging findings were patchy as compared with confluent white matter abnormalities seen in most patients with MNGIE. The normal plasma thymidine level in our patient prompted us to look for other molecular explanations of her disease. When mitochondrial depletion was demonstrated in her muscle, we extended the sequencing to genes associated with mtDNA depletion syndromes. Identification of RRM2B mutations in a patient with classic features of MNGIE expands the molecular repertoire of this genetic disorder. The role of RIR2B in de novo deoxynucleotide triphosphate synthesis suggests that there could be multiple mechanisms resulting in MNGIE phenotype and that depletion of the intramitochondrial nucleotide pool is the common biochemical abnormality underlying the neurological abnormalities in this condition.

CONCLUSIONS

We conclude that mutations in RRM2B may result in a phenotype clinically similar to MNGIE. Therefore, RRM2B sequencing should be considered in patients with MNGIE with normal thymidine levels. Development of symptoms in midadulthood expands the spectrum of age at presentation in RRM2B-related disorders. Aminoglycosides should be cautiously used in patients with severe gastrointestinal dysmotility as this can be the first clinical presentation of MNGIE.

Accepted for Publication: March 24, 2009.
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Financial Disclosure: None reported.

Funding/Support: Dr Lichtarge is supported by grants R01-GM066099 and R01-GM079656 from the National Institutes of Health. Dr Katsonis has a fellowship from the Pharmacoinformatics Training Program of Keck Center of the Gulf Coast Consortium (grants 5 R90 DK071505-03 and 5 R90 DK071505-04 from the National Institutes of Health); the program is supported by a National Institutes of Health–funded training grant.
Additional Contributions: Amina Jinna, MD, Shweta Dhar, MD, Richard Lewis, MD, Kerri Lamance, RN, and the Baylor College of Medicine Mitochondrial Laboratory contributed to the caring management of our patient. We thank the patient’s family for their kind cooperation.

REFERENCES


Announcement

Calendar of Events: A New Web Feature. On the new Calendar of Events site, available at http://pubs.ama-assn.org/cgi/calendarcontent and linked off the home page of the Archives of Neurology, individuals can now submit meetings to be listed. Just go to http://pubs.ama-assn.org/cgi/cal-submit/ (also linked off the Calendar of Events home page). The meetings are reviewed internally for suitability prior to posting. This feature also includes a search function that allows searching by journal as well as by date and/or location. Meetings that have already taken place are removed automatically.