C. Fratter, MPhil
P. Raman, MSc
C.L. Alston, BSc
E.L. Blakely, PhD
K. Craig, PhD
C. Smith, PhD
J. Evans, PhD
A. Seller, PhD
B. Czermin, PhD
M.G. Hanna, FRCP
J. Poulton, DM,
FRCPCH
C. Brierley, PhD, FRCP
T.G. Staunton, MD,
FRCP
P.D. Turnpenny, FRCP
A.M. Schaefer, MRCP
P.F. Chinnery, PhD,
FRCP
R. Horvath, MD
D.M. Turnbull, PhD,
FRCP
G.S. Gorman, MRCP
R.W. Taylor, PhD

RRM2B MUTATIONS ARE FREQUENT IN FAMILIAL PEO WITH MULTIPLE mtDNA DELETIONS

Disorders of mitochondrial DNA (mtDNA) maintenance leading to multiple mtDNA deletions are a significant cause of inherited neurologic disease in adults, but the underlying nuclear gene defects remain elusive in many patients. Following the recent description of a truncating mutation in the *RRM2B* gene—encoding the small subunit, p53R2, of the p53-inducible ribonucleotide reductase protein—in 2 families with autosomal-dominant progressive external ophthalmoplegia (adPEO),¹ we determined the frequency of *RRM2B* mutations in a large cohort of patients with PEO and multiple mtDNA deletions in muscle in whom mutations in all known candidate genes (e.g., *POLG*, *POLG2*, *SLC25A4*, and *PEO1*) had been excluded.²

Methods. We studied 75 unrelated probands with PEO, a mosaic defect of cytochrome *c* oxidase (COX) activity, and multiple mtDNA deletions in skeletal muscle who had been referred to Mitochondrial Diagnostic Centers at Newcastle, Oxford, or Munich for clinical assessment and histologic/molecular genetic analysis. The entire coding region, including intron–exon boundaries, of the *RRM2B* gene was determined as previously described.¹ *RRM2B* exon copy number (exons 1–8) was assessed by MLPA (MRC-Holland kit P089-A1) in patients with single, heterozygous missense mutations.

Standard protocol approvals, registrations, and patient consents. This study was approved and performed under the ethical guidelines issued by each institution for clinical studies, with written informed consent obtained from all subjects.

Results. We identified 10 different *RRM2B* variants in 12 subjects, representing 16% of our undiagnosed cohort. The table summarizes these, together with details of the patients' clinical presentation. All 10 *RRM2B* changes were novel and were absent in 352 control alleles. All 7 missense variants altered amino acids conserved across mammalian species, and all except p.Gly273 were conserved to at least *C. elegans* (figure e-1 on the *Neurology*[®] Web site at www.neurology.org).

Two patients (group A, table) harbored compound heterozygous, missense variants implying autosomal recessive inheritance. Seven patients (group B) harbored single heterozygous, truncating mutations within exon 9 (figure e-2), all of whom had family histories consistent with autosomal dominant inheritance together with supportive genetic segregation data in one family (patient 4). We detected single heterozygous, missense variants in the 3 patients in group C (MLPA excluded exonic copy number

variation in *trans*); however, the pathogenicity of these 3 variants is provisional in the absence of further supporting evidence.

Discussion. Our data confirm a previous report describing dominant *RRM2B* mutations as an important cause of mtDNA maintenance disorders in adults¹ and provide the first description of recessive *RRM2B* mutations associated with multiple mtDNA deletions and respiratory chain deficiency.

The clinical spectrum of disease associated with *RRM2B* gene mutations ranges from a fatal infantile neuromuscular syndrome with renal tubular insufficiency^{3–6} to late-onset PEO.¹ Early-onset mtDNA depletion syndromes due to recessive *RRM2B* mutations are characterized by myopathy and renal proximal tubulopathy, often exhibit multisystem involvement, and are invariably fatal in early childhood.^{3–6} CNS features may include seizures,^{3,6} hearing loss,^{4,5} microcephaly, and global developmental delay.⁴ Respiratory insufficiency^{3,4,6} and gastrointestinal dysmotility^{3,4,6} are not infrequent. Our data confirm that adult-onset adPEO due to *RRM2B* mutations is associated with a more benign myopathic phenotype and characterized by muscle-restricted, mtDNA deletions. Ptosis and ophthalmoparesis are the predominant clinical characteristics of *RRM2B* defects in our adult-onset cohort accompanied by mild muscle-related symptoms such as fatigue, bulbar dysfunction, and proximal muscle weakness. In contrast, the 2 childhood onset recessive cases display a variable spectrum of clinical features.

The 2 families previously reported with PEO and multiple mtDNA deletions due to *RRM2B* mutations were both found to have the same exon 9 truncating mutation, p.Arg327X.¹ We identified 3 different truncating mutations in exon 9 (p.Leu317X, p.Glu318X, and p.Asn322LysfsX4) in 7 cases (group B), thereby establishing this class of mutation as an important cause of adPEO. Two further *RRM2B* mutations—p.Asn307IlefsX11 and p.Leu317Val—have previously been implicated in recessive mtDNA depletion, highlighting exon 9 as a mutation hotspot in mtDNA maintenance disorders.⁴

Mutations in *POLG* remain the major cause of multiple mtDNA deletion disorders, accounting for some 25% of all patients with mitochondrial disease presentations,⁷ followed by dominant *PEO1* mutations (~15% of our PEO/multiple mtDNA deletion cohort).² We have now identified *RRM2B* mutations in ~9% of patients, since the undiagnosed cohort studied here constitutes ~55% of our total cohort with PEO and multiple mtDNA deletions. Consequently, mutations of *RRM2B* are more common than *OPA1*, *SLC25A4*, and *POLG2* mutations,

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Table Clinical and molecular genetic findings in patients with RRM2B mutations

Patient	Age at onset, y	Current age, y	Family history (affected relatives) ^a	Clinical features	RRM2B cDNA change	Amino acid change
Group A, autosomal recessive (no family history)						
1	Birth	Died age 25	None	PEO, ptosis, myopathy, renal failure, deaf, developmental delay, hypoparathyroidism, hypogonadism, areflexia, dysphagia	c.431C>T and c.632G>A	p.Thr144Ile and p.Arg211Lys
2	4	14	None	PEO, ptosis, deaf	c.606T>A and c.817G>A	p.Phe202Leu and p.Gly273Ser
Group B, autosomal dominant						
3	30	52	Mother	PEO, ptosis	c.950delT	p.Leu317X
4	53	73	Father, brother, paternal nephew ^b	Severe PEO, ptosis, mild dysphagia, fatigue, ataxia, glaucoma	c.950delT	p.Leu317X
5	46	66	Mother	Severe PEO, ptosis, proximal myopathy, ataxia	c.950delT	p.Leu317X
6	53	64	Mother	PEO, ptosis, dysarthria, dysphagia, fatigue	c.952G>T	p.Glu318X
7	30	62	Brother, father, grandfather	PEO, fatigue, diabetes, GI symptoms	c.965dupA	p.Asn322LysfsX4
8	54	64	Mother, sister	PEO, ptosis, dysphagia, fatigue	c.965dupA	p.Asn322LysfsX4
9	26	61	Maternal grandmother and aunt	PEO, ptosis, dysphagia, fatigue, proximal myopathy, dysphonia, IBS	c.965dupA	p.Asn322LysfsX4
Group C, single heterozygous missense change						
10	59	71	None	PEO, no ptosis, encephalopathy, stroke-like episodes, deaf, cognitive decline	c.122G>A	p.Arg41Gln
11	50	75	Son ^c	PEO, ptosis, deaf, dysphonia, proximal myopathy, ataxia, cataracts, glaucoma	c.583G>A	p.Gly195Arg
12	15	25	None	Severe PEO, ptosis, dysphagia, dysphonia, fatigue, ataxia, proximal myopathy, deaf, facial weakness, IBS	c.686G>T	p.Gly229Val

Abbreviations: GI = gastrointestinal; IBS = irritable bowel syndrome; PEO = progressive external ophthalmoplegia.

^a Historical reports of affected relatives; formal genetic testing not performed unless indicated (relatives of patient 4 and patient 11).

^b c.950delT (p.Leu317X) was shown to segregate with disease in patient 4 and other clinically affected relatives indicated above, all of whom had PEO although muscle biopsy was not performed.

^c c.583G>A (p.Gly195Arg) was shown to segregate with disease in patient 11 and his son, although the son's symptoms were mild and a muscle biopsy had not been performed.

which together comprise less than 5% of our patient cohort, and as such sequencing of this gene should be considered in the diagnostic algorithm for multiple mtDNA deletion disorders once *POLG* and *PEO1* mutations have been excluded.

From the Oxford Medical Genetics Laboratories (C.F., C.S., J.E., A.S.), Churchill Hospital, Oxford; Mitochondrial Research Group, Institute for Ageing and Health (P.R., C.L.A., E.L.B., K.C., A.M.S., P.F.C., R.H., D.M.T., G.S.G., R.W.T.), and Institute of Genetic Medicine (P.F.C., R.H.), Newcastle University, Newcastle upon Tyne, UK; Medical Genetic Centre (B.C.), Munich, Germany; UCL MRC Centre for Neuromuscular Diseases (M.G.H.), Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London; Nuffield Department of Obstetrics and Gynaecology (J.P.), University of Oxford, Oxford; Department of Neurology (C.B.), The West Suffolk Hospital, Bury St Edmunds, Suffolk; Department of Neurology (T.G.S.), Norfolk and Norwich University Hospital, Norwich; and Department of Clinical Genetics (P.D.T.), Royal Devon and Exeter Hospital, Exeter, UK.

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Address correspondence and reprint requests to Professor R.W. Taylor, Mitochondrial Research Group, Institute of Ageing and Health, The Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK; r.w.taylor@ncl.ac.uk

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