Severe childhood SMA and axonal CMT due to anticodon binding domain mutations in the *GARS* gene

Abstract—We screened 100 patients with inherited and sporadic lower motor neuron degeneration and identified three novel missense mutations in the glycyl-tRNA synthetase (*GARS*) gene. One mutation was in the anticodon binding domain and associated with onset in early childhood and predominant involvement of the lower limbs, thus extending the phenotype associated with *GARS* mutations.

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P.A. James, MD; M.Z. Cader, DPhil; F. Muntoni, FMedSci; A.-M. Childs, MD; Y.J. Crow, PhD; and K. Talbot, DPhil

There is considerable clinical and genetic overlap between subtypes of inherited neuropathy.[1] Mutations in the glycyl-tRNA synthetase (*GARS*) gene have been found in families with Type V (upper limb predominant) autosomal dominant distal hereditary motor neuropathy (HMN)/spinal muscular atrophy (SMA), and also in Charcot–Marie–Tooth disease (CMT) Type 2D, in which there is similar distribution of weakness but additional evidence of sensory involvement.[2] These apparently distinct phenotypes are part of a spectrum of conditions caused by *GARS* dysfunction.[3]

We undertook a mutation screen of the *GARS* gene in a cohort of patients with dominant or sporadic SMA of diverse types to investigate whether the phenotypic spectrum extends outside typical dSMA/HMN Type V.

Methods. We screened 100 patients diagnosed with distal SMA, HMN, or motor axonal CMT for mutations in *GARS*. PCR primers were designed to amplify each exon including splice sites. Amplicons were analyzed using denaturing high-performance liquid chromatography (DHPLC) (Transgenomic WAVE). Fragments with abnormal patterns suggesting heteroduplex formation were sequenced using the dye terminator method on an ABI Prism 3700 sequencer (Applied Biosystems).

Results. We identified three novel mutations.

Family 1: G598A (2313g→c). The proband was born at full term by uncomplicated vaginal delivery to unrelated parents with no family history of weakness. Early motor milestones were normal, achieving head control at age 3 months and sitting unaided at 6 months. However, her parents noticed at this stage that both of her feet were "floppy." She was using a walking frame by age 18 months but never achieved independent walking. At age 3 years, she had reduced tone in the limbs, absent tendon reflexes, and distal muscle weakness and wasting in the legs and hands. Proximal muscle strength was preserved. Results of motor nerve conduction velocities and sensory studies were normal, and EMG and muscle biopsy were consistent with denervation due to anterior horn cell degeneration. SMN1 gene testing results were normal. By age 7 years, her condition had progressed with marked distal weakness and wasting in the legs (figure) and additionally hip extension (Medical Research Council [MRC] Grade 2) and flexion (MRC Grade 3). Wasting and weakness of the small muscles of the hand (figure) was associated with impaired finger grip. There was mild weakness of eye closure and a weak cough, and her voice was decreased in volume, but there was no tongue wasting. She was using accessory muscles of respiration, and forced vital capacity was reduced to 80% predicted, with no overt diaphragmatic weakness. Bilateral leg flexion contractures had developed at the hip, and she had a marked lumbar lordosis and scoliosis (figure). Both parents were healthy and did not carry the mutation, which we presume arose de novo in the proband.

Family 2: S581L (2260c→t). The proband is a woman who presented to orthopedic services at age 27 years with foot deformity that began in late adolescence. Referral to a neurologist and subsequent neurophysiologic analysis led to a diagnosis of CMT2. There is an extensive family history spanning four generations, including the proband's grandmother, who is still alive at age 97 years. At the time of her own diagnosis, she became aware that her 4-year-old son was affected because of uneven wearing of the soles of his shoes and a mildly abnormal gait. Despite this, he was able to participate in sport at school. By age 13 years, his foot deformity required surgical intervention, and throughout adolescence, his gait deteriorated. Now aged 30 years, he can walk and manage stairs slowly, but he trips frequently. Both mother and son have severe distal weakness (MRC Grade 2 to 3) and wasting, more severe in the lower than upper limb, and areflexia and reduced vibration and joint position sense at the ankles, consistent with a diagnosis of CMT2.

Family 3: I280F (1358a→t). The proband is a woman now aged 34 years who presented to an orthopedic surgeon at age 11 years with bilateral weakness of the feet. At presentation, there was wasting of the first dorsal interossei. Her father, grandfather, and two siblings were also affected with an age at onset from the mid-teens. Her son presented with foot drop and mild hand-grip weakness at

From the Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK (P.A.J., M.Z.C., K.T.); Dubowitz Neuromuscular Centre, Imperial College London, London, UK (F.M.); Department of Pediatric Neurology, Leeds General Infirmary, Leeds, UK (A.-M.C.); and Yorkshire Regional Genetic Service, St James's University Hospital, Leeds, UK (Y.J.C.).

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Address correspondence and reprint requests to Dr. Kevin Talbot, Henry Wellcome Building for Gene Function, Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 1LF, UK; e-mail: kevin.talbot@clneuro.ox.ac.uk

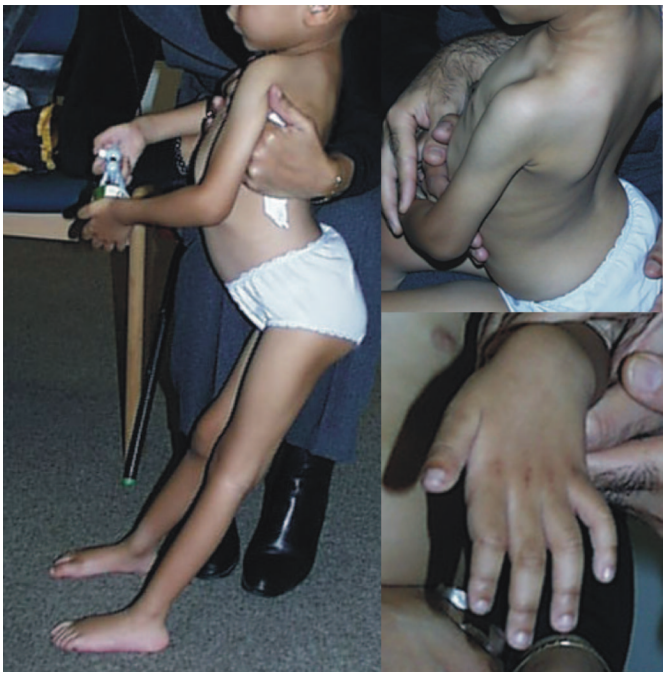

Figure. Clinical features of most severely affected patient showing generalized muscle wasting, marked wasting of small muscles of hand, and increased lordosis and scoliosis of spine.

age 11, associated with wasting of the hands and pes cavus. Sensory testing was clinically and neurophysiologically normal, but compound muscle action potential amplitudes were reduced by 20% in the upper limb and 50% in the lower limb. Both individuals are ambulant, with occasional falls.

Discussion. The spinal muscular atrophies, or hereditary motor neuronopathies, are a clinically and genetically heterogeneous group of disorders characterized by primary degeneration of the anterior horn cells of the spinal cord and sometimes of the bulbar motor nuclei. The distal forms are said to account for 10% of SMA[4] but in our experience are commoner. Distal HMN Type V, with a characteristic picture of onset in adolescence, slow progression, and predominant upper limb involvement, and CMT2D, a similar disorder with minor sensory involvement, are allelic disorders due to mutations in the gene for glycyl-tRNA synthetase.[2,3,5]

In this study, we screened a highly heterogeneous population of patients with inherited and sporadic forms of lower motor neuron degeneration to determine whether *GARS* mutations cause a broader phenotype than previously described. We have identified three new mutations. One family carrying an I280F mutation has a phenotype consistent with previously described dHMNV families, with onset at age 11 years and early upper limb involvement. In contrast, two further families with mutations in the anticodon binding domain presented with predominant lower limb involvement in infancy or early childhood. In particular, we identified a de novo dominant G598A mutation located in the center of the anticodon binding domain, associated with a severe form of infantile SMA affecting the lower limbs more severely, with failure to acquire ambulation and associated respiratory and facial muscle involvement.

Table *Comparison of patients with GARS* mutations*

Mutation	Reference	Age at onset	Distribution	Diagnosis
S581L	This study	4 y	LL > UL	CMT2
G598A	This study	6 mo	LL > UL	Infantile SMA
I120F	This study	11–18 y	LL = UL	Distal HMN
L129P	2	17 y	UL > LL	Distal HMN
G240R	2	16–30 y	UL > LL	CMT2
E71G	2	18 y	UL > LL	Distal HMN or CMT2
G526R	2	13 y	UL > LL	Distal HMN
H418R	3	?	UL > LL	Distal HMN
D500N	5	10–50 y	UL > LL	Distal HMN or CMT2

* Previously described mutations have been reported in families with slowly progressive upper limb predominant disease.

GARS = glycyl-tRNA synthetase; LL = lower limb; UL = upper limb; CMT = Charcot–Marie–Tooth disease; SMA = spinal muscular atrophy; HMN = hereditary motor neuropathy.

Previously reported mutations (see table) cluster around residues of the catalytic core[2] and are expected to affect the enzyme kinetics, although this remains to be formally demonstrated. The anticodon binding domain is important for discriminating the correct tRNA to ligate with glycine. Human *GARS* is a homodimer, and it possible that the G598A mutation exerts a dominant negative effect severely reducing enzymatic activity, or that phenotypic severity correlates with functions other than enzyme efficiency. The specificity of motor neuron degeneration due to mutations in glycyl-tRNA synthetase is unexplained. Aminoacyl-tRNA synthetases have a ubiquitous cellular expression and are considered to be among the most phylogenetically ancient of proteins, with a pivotal role in ensuring the strict fidelity of translation of the genetic code to protein by ligating amino acids to their cognate tRNA. *GARS* is unusual among the synthetases in that the mitochondrial and cytoplasmic enzymes are encoded by the same gene with two alternative translation starts. Aminoacyl-tRNA synthetases also have important noncanonical functions, including cell-cycle regulation and signal transduction,[6] which are often idiosyncratic to a particular ARS, suggesting that these enzymes have been repeatedly targeted for evolutionary adaptation. Specifically, *GARS* has been shown to participate in 3′ end mRNA formation in yeast.[7] The recent identification of mutations in tyrosyl-tRNA synthetase in patients with an axonal form of CMT[8] suggests that these proteins may share a noncanonical neuron-specific function in cell maintenance.

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Microhemorrhages in a patient with reversible posterior leukoencephalopathy syndrome

H. Kawai, MD, PhD; H. Nakamura, MD; M. Sanada, MD, PhD; S. Kitahara, MD, PhD; and K. Maeda, MD, PhD, Shiga, Japan

A 67-year-old man presented with convulsion and cortical blindness. His past history was remarkable for untreated hypertension, and his blood pressure was 230/110 mm Hg. Axial fluid-attenuated inversion recovery image showed symmetric hyperintense lesions in the posterior white matter (figure, A and B). The T2*-weighted MR image displayed marked microhemorrhages widespread throughout, exclusively in posterior circulation (figure, C and D). His visual acuity returned to normal on the second hospital day. The pathologic features of posterior reversible leukoencephalopathy characterized by cerebral edema and petechial hemorrhages[1] were clearly demonstrated by T2*-weighted MR image[2] in a real time manner.

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Address correspondence and reprint requests to Dr. H. Kawai, Division of Neurology, Department of Medicine, Shiga University of Medical Science, Seta, Otsu, Shiga 520-2192, Japan; e-mail: hirok@belle.shiga-med.ac.jp

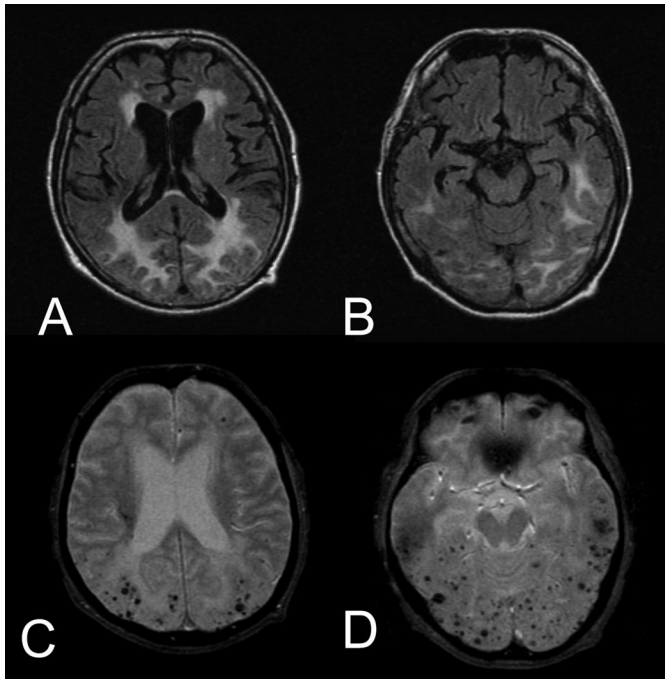

Figure. (A, B) Fluid-attenuated recovery images show vasogenic edema in occipital white matter. (C, D) T2-weighted MR images show multiple microhemorrhages in a posterior circulation distribution.*