LETTERS TO THE EDITOR

A novel de novo point mutation in the GTP cyclohydrolase I gene in a Japanese patient with hereditary progressive and dopa responsive dystonia

Hereditary progressive dystonia is characterised by lower limb dystonia of childhood onset with marked diurnal fluctuation and shows a dramatic and stable response to low dose levodopa. The disease is transmitted in autosomal dominant inheritance, and Segawa *et al*¹ proposed hereditary progressive dystonia as a new disease entity in the early 1970s. Dopa responsive dystonia, which was first proposed by Nygaard *et al*² in 1988, is essentially identical to hereditary progressive dystonia although it may include some other heterogeneous dystonias. The GTP cyclohydrolase I (GTP-CH I) gene on chromosome 14 is the causative gene of hereditary progressive/dopa responsive dystonia³ and more than 20 different mutations have been reported. We report a novel non-sense mutation in the GTP-CH I gene in a genetically confirmed sporadic Japanese patient.

A 12 year old Japanese girl developed gait disturbance with a dystonic pes equinovarus posture in the right leg at the age of 4 years. These symptoms showed diurnal fluctuation; they were mild in the morning and worsened later in the day, and sleep improved the symptoms. They dramatically and continuously responded to low dose levodopa/

carbidopa (100 mg/day) without adverse effects. Other members of her family, comprising the parents and one sister, had no symptoms.

Blood specimens of the patient and of the parents and sister were available for genetic analysis. For mutation analysis, genomic DNA was extracted from EDTA anticoagulated peripheral blood. Fragments of DNA containing the entire coding region of the GTP-CH I gene were obtained from genomic DNA by polymerase chain reaction (PCR) according to the method of Ichinose *et al*.³ Direct nucleotide sequencing of PCR products was performed with an automated DNA sequencer (Applied Biosystems 310) using the same primers as were used for amplification.

Direct nucleotide sequencing of genomic DNA of the patient showed a G to A transversion in exon 1 of the GTP-CH I gene (data not shown). This mutation produces a substitution of the tryptophan residue (TGG) with a stop codon (TGA) at position 96, which creates a new Eco57I cleavage site. The sequencing of the parents and the sister of the patient showed no mutation. To confirm the mutation, exon 1 was amplified and digested by Eco57I in all the four subjects. The restriction fragment length polymorphism consisted of two fragments (370 and 132 bp) in the mutant allele and one fragment (502 bp) in the normal allele (figure). The restriction pattern in the patient was consistent with heterozygous status, consisting of one each of the mutant and normal alleles, whereas that of the parents and sister, with homozygous status of two normal alleles. No other mutations were detected in the coding region of the gene.

The patient presented with typical clinical features of hereditary progressive dystonia. A new mutation in the GTP-CH I gene of the present patient causes a stop codon, and this mutation is most likely the pathogenic mutation. GTP-CH I catalyses the initial and rate limiting steps of tetrahydrobiopterin synthesis. Tetrahydrobiopterin is an essential cofactor for tyrosine hydroxylase, the rate limiting enzyme in the dopamine synthesis pathway. GTP-CH I activity in patients with hereditary progressive dystonia is less than 20% of that in normal subjects.³ The non-sense mutation in exon 1 confirmed in the present patient would have caused premature truncation of the GTP-CH I protein with a loss of an estimated 60% of the amino acid residues from the C terminal, and GTP-CH I activity, though it was not measured, may have been reduced below the critical threshold. To date, 22 different mutations in the GTP-CH I gene have been identified worldwide, and there seems to be no evidence of the founder effect.

To our knowledge, only a single case (patient 2 of Furukawa *et al*)⁴ was confirmed to be genetically sporadic. They identified the G to A transversion at the splice acceptor site of intron 1 in the GTP-CH I gene, causing skipping of the entire exon 2 in the mature mRNA. In the present family, the mutation was confirmed in the proband only, and not in the parents and the sister. The restriction fragment length polymorphism generated by Eco57I consisted of one fragment (502 bp) common to the parents and sister and an additional two fragments (370 and 132 bp) unique to the patient. The present patient was thus confirmed to be genetically sporadic and heterozygous, and is the second report of de novo mutation in the GTP-CH I gene.

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Central nervous system involvement in four patients with Charcot-Marie-Tooth disease with connexin 32 extracellular mutations

In a recent issue of this *Journal*¹ there was a report of two cases of multiple sclerosis with duplicated CMT1A. Here we present four additional patients with Charcot-Marie-

(A) Pedigree of a Japanese hereditary progressive/dopa responsive dystonia family with mutation in the GTP-CH I gene. The affected proband is represented by solid symbol, and unaffected family members, by open symbols. (B) Agarose gel electrophoresis of Eco57I restriction pattern in the exon 1 of GTP-CH I gene. PCR product (502 bp) including exon 1 of the GTP-CH I gene from the patient was partially digested into two fragments of smaller sizes (370 and 132 bp) due to a Eco57I cleavage site created by a mutation in exon 1 of the gene. The 502 bp fragments from the unaffected family members remained undigested. Lane M=pUC19 DNA MspI digestion marker.

Tooth disease with CNS involvement as shown by electrophysiological studies and the presence of myelin lesions in brain MRI. In our patients point mutations were identified in GJB1, a gene coding for connexin 32 (GenBank acc number 117688). We also point to the possible importance of the position (intracellular or extracellular) of the mutations in the involvement or not of the CNS.

In the process of investigating a panel of patients with Charcot-Marie-Tooth disease for mutations leading to the disease, we have screened the GJB1 gene for mutations in six patients (C12, C64, C10, and C13 (all unrelated), and C2-1 and C2-2 (brothers) who did not have a CMT1A duplication and had a family history compatible with an X linked mode of transmission. GJB1 is a gene coding for connexin 32 (Cx32), a gap junction protein that is found in both the peripheral and the central nervous systems² and that has been reported by many to be responsible for CMTX, the X linked subtype of Charcot-Marie-Tooth disease. We performed our search using SSCP and nucleotide sequencing when additional bands were detected.³ Mutations leading to amino acid sequence changes and transmitted with the disease were detected in all six patients, whereas these nucleotide variations were not detected in 150 healthy control X chromosomes. Because Cx32 is a protein expressed in both the peripheral and the central nervous systems we proceeded to test for CNS involvement in those patients using electrophysiological (table) and MRI techniques. The clinical and laboratory findings for our patients were as follows.

Patient C2-1, a 19 year old man, had a history of 5 hour to 3 day long episodes of weakness—generalised or at other times localised to the left or right half of the body—dysarthria, and difficulty in swallowing. Neurological clinical examination showed muscle weakness and peripheral atrophy of the limbs, absence of tendon reflexes, distal loss of vibration in the legs, indifferent plantar response, and bilateral pes cavus. The CSF was normal. Brain SPECT (Tc-99m HMPAO {15 mCi}) showed significant reduction of blood flow in the left parietal lobe. Brain MRI showed bilateral symmetric areas in the central white matter supraventricularly with high intensity signal in T2 weighted

images. Electrophysiological studies (table) showed that both the central and peripheral nervous systems were affected. The patient had a C to T transition at position 164 (novel mutation).

Patient C2-2, the 21 year old brother of C2-1, had the same symptoms (multiple episodes of muscle weakness) since the age of 10 years. The clinical findings were also the same. In this case the CSF showed IgG oligoclonal bands. Brain MRI was normal. Electrophysiological studies also showed that both the central and peripheral nervous systems were affected. The patient had the same mutation as his brother C2-1, as was expected.

Patient C12, a 63 year old man, had progressive muscle weakness and peripheral atrophy of the extremities since the age of 9 years. When admitted he had severe muscle weakness, absence of tendon reflexes, distal loss of vibration in the legs, dysmetria, fine tremor, positive Romberg's sign, and bilateral pes cavus. The CSF showed an IgG index of 1.0 (normal<0.85) and oligoclonal bands. Brain MRI showed foci of demyelination in the white matter of the cerebral hemispheres. Electrophysiological studies also showed that both the central and peripheral nervous system were affected. The patient had the same mutation as patient C2-1.

Patient C64, a 58 year old man, presented with progressive muscle weakness and peripheral atrophy since the age of 10 years. His CSF was not sampled. Brain MRI showed high density areas of demyelination in the white matter on the left side in the frontoparietal area. The patient had a G to A transition at position 491 (novel mutation).

Patient C10, a 78 year old man, presented with progressive muscle weakness and peripheral atrophy of the legs since the age of 11. The clinical picture was the same as that of patient C64. The CSF and brain MRI were normal. The patient had a T to A transition at position 359 (novel).

Patient C13, a 40 year old man, presented with progressive muscle weakness and peripheral atrophy starting at the age of 7 years. His CSF and brain MRI were normal. The patient had a C to T transition at position 43 (already reported⁴). Sural nerve biopsy performed in patients C2-1, C2-2, C64, and C10 showed loss of muscle fibres and "onion bulb" formation. It is of particular interest,

possibly implying an underlying mechanism, that the four patients with evidence of CNS involvement (C2-1, C2-2, C12, and C64) had mutations in the extracellular domain of Cx32, and by contrast, in the two patients with no evidence of CNS involvement (C10, C13) the mutations were in the intracellular domain of the protein.

The exact pathogenetic mechanisms of the various types of myelin damage are not yet fully understood. Therefore, we cannot reach any convincing conclusions about the possible relations between those degenerative processes and the position of Cx32 mutations. However, we hope that our findings will contribute to a better understanding of some myelin damage mechanisms.

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Lack of mutation G209A in the α -synuclein gene in French patients with familial and sporadic Parkinson's disease

Polymeropoulos *et al* described the genetic linkage of a large Italian Parkinson's disease pedigree (Contursi) to chromosome 4q21-q23¹; more recently the sequence of the α -synuclein gene located in this chromosomal region in affected patients from this kindred disclosed that most of the affected members were heterozygous for a missense mutation (alanine→threonine) at position 53 of the protein.² This same missense mutation was also found in three additional unrelated families of Greek origin with Parkinson's disease.

The mutation was found by DNA sequence analysis of the fourth exon of the α -synuclein gene, disclosing a single base pair change at position 209 from G to A (G209A), and creating a novel *Tsp45I* restriction site. Using a polymerase chain reaction (PCR) assay and *Tsp45I* restriction, we have found some French patients with Parkinson's disease. Genomic DNA was amplified with primers 3 and 13 of the DNA sequence (GenBank ID U46898) in a 50 μ l reaction volume (10 mM Tris-HCl, pH=8.3; 50 mM KCl; 1.5 mM MgCl₂; 200 μ M of each dNTP and 1 U *Taq* polymerase); cycling parameters were: one initial step of hybridisation at 94°C during 5 minutes, 35 cycles of PCR (hybridisation at 60°C, 40 seconds, extension at 72°C, 40 seconds; denaturation at 94°C, 40 seconds) and a final extension step (5 minutes at 74°C). A 20 μ l aliquot of the 216 base pair (bp) product was restricted with the

Electrophysiological data from the six patients

		C2-1	C2-2	C12	C64	C10	C13	Normal
MNC:								
Median	Ampl (mV)	4	0.8	0.6	0.8	1.0	0.2	
	DL (ms)	4.6	4.4	6.6	5.7	4.8	8.6	
	CV (m/s)	36	33	29	31	29	21	
Peroneal	Ampl (mV)	2.5	0.4	NE	NE	NE	NE	
	DL (ms)	7.4	5.1	NE	NE	NE	NE	
	CV (m/s)	38	30	NE	NE	NE	NE	
SNC:								
Sural	Ampl (mV)	2	4	NE	0.5	NE	NE	
	DL (ms)	3.6	2.9	NE	8.1	NE	NE	
	CV (m/s)	31	31	NE	22	NE	NE	
BAERs:								
Right	I (ms)	1.58	2.20	1.82	1.68	1.52	1.41	<1.73
	III (ms)	4.54	4.58	4.63	4.61	3.12	2.89	<3.79
	V (ms)	6.14	7.22	6.87	6.24	5.25	4.97	<5.72
Left	I (ms)	1.72	1.62	1.98	1.85	1.43	1.51	<1.73
	III (ms)	4.24	4.60	4.75	4.79	2.98	3.13	<3.79
	V (ms)	6.20	6.22	7.31	6.87	4.95	4.87	<5.72
VEPs:								
Right	Lat (ms)	111.5	95.5	NP	112.2	NP	NP	80-108
	Lat (ms)	97	110	NP	110.8	NP	NP	R-L<8

MNC=Motor nerve conduction; Ampl=amplitude; DL=distal latency; CV=conduction velocity; SNC=sensory nerve conduction, BAERs=brain stem auditory evoked responses: waves I, III, and V; VEPs=visual evoked potentials; Lat=latency; NE=not evoked; NP=not performed.