

# The phenylalanine loading test in the differential diagnosis of dystonia

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**Abstract**—Early diagnosis of dopa-responsive dystonia (DRD) and its delineation from other dystonic syndromes is of great relevance because DRD is an eminently treatable condition. The possible relevance of the phenylalanine loading test (Phe-L) in differentiating DRD from primary focal and generalized dystonia was investigated. A marked difference in the phenylalanine/tyrosine ratio between patients with DRD and patients with other types of dystonia was observed. This indicates that Phe-L may be helpful in the differential diagnosis of dystonias.

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There is excellent and sustained improvement on simple oral drug treatment with the dopamine precursor substance L-dopa in patients with dopa-responsive dystonia (DRD), unlike all other forms of dystonia. However, there is considerable phenotypic overlap between DRD and other dystonic syndromes.<sup>1</sup> Thus, a diagnostic test that would reliably identify DRD and delineate patients with DRD from those with other dystonic syndromes would be of considerable clinical relevance to ensure early treatment of DRD while avoiding unnecessary L-dopa treatment in patients with other forms of dystonia.

In 1994, the GTP cyclohydrolase I gene (*GCH-1*) was isolated as the first causative gene for DRD and the first mutations in classic cases of DRD were identified.<sup>2</sup> However, sequence analysis of *GCH-1* is not useful as a routine diagnostic procedure because mutations in *GCH-1* can only be found in approximately half of all DRD cases.<sup>3</sup> Furthermore, unlike in the *DYT1* gene *torsinA*, there is no common mutation. Rather, the mutations are spread over the entire coding sequence and the adjacent splice sites. Mutations outside the coding region might also be of some relevance and there may also be genetic heterogeneity in DRD.<sup>1,4,5</sup> Thus, mutation screening in suspected cases of DRD is time-consuming and does not reliably lead to confirmation of the diagnosis.

Outside the CNS, BH4 also acts as a cofactor for phenylalanine hydroxylase, which catalyzes the hydroxylation of phenylalanine to tyrosine in the liver. Autosomal recessively inherited, complete or near-complete BH4 deficiency presents in early infancy with hyperphenylalaninemia in addition to severe impairment of neurotransmitter synthesis.<sup>6</sup>

An abnormal ratio of phenylalanine to tyrosine in

the group analysis of the so-called phenylalanine loading test (Phe-L) compared to normal controls has previously been described in a group of patients with genetically proven DRD with classic phenotype from two large families, indicating that phenylalanine metabolism is also comprised in DRD.<sup>7</sup> However, information is lacking as to whether the Phe-L might be useful to differentiate DRD in an individual patient from other types of dystonia that overlap clinically with DRD, such as focal or generalized idiopathic torsion dystonia (F-ITD, G-ITD). The aim of this study was to investigate the possible relevance of the Phe-L in differentiating DRD from F-ITD or G-ITD. Furthermore, the previously reported protocol of the Phe-L involves repeated blood taking and measurements of phenylalanine and tyrosine at five time points (0, 1, 2, 4, and 6 hours). A reduced number of measurements would facilitate the introduction of the Phe-L into routine clinical practice and we therefore investigated whether single time point analysis might be sufficient.

**Patients and methods.** *Patients.* In total, 30 patients were investigated. Ten patients had DRD, 10 had F-ITD, and 10 had G-ITD. The mean ages in the different patient groups were 35.3 years (SD 11.9 years) for the DRD patients, 59.8 years (SD 15.8 years) for F-ITD, and 35.8 years (SD 11.4 years) for G-ITD. The mean age at onset was 4.1 years (SD 3.1 years) for DRD, 48.1 years (SD 11.7 years) for F-ITD, and 24.3 years (SD 12.7 years) for G-ITD. The sex distribution was as follows: DRD: nine women, one man; F-ITD: nine women, one man; G-ITD: seven women, one man.

*Controls.* A total of 39 controls with a mean age of 29.8 years (SD 17.0 years) were included.

Mutations in the *GCH-1* gene were detected in 7 of the 10 patients with DRD; none of the patients with F-ITD or G-ITD had the typical three-base pair deletion in the *DYT1* gene *torsinA* (data not shown).<sup>8</sup> Secondary causes of dystonia such as Wilson disease or intracranial lesions were excluded in all patients. The diagnosis of DRD in the mutation-negative cases was based on a

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**Table** Phenylalanine to tyrosine ratios before and 1, 2, and 4 hours after the phenylalanine loading (phe-load)

Timing	DRD	F-ITD	G-ITD	Controls
Before phe-load	1.28 ( $\pm 0.51$ )	1.14 ( $\pm 0.82$ )	0.98 ( $\pm 0.17$ )	0.88 ( $\pm 0.32$ )
1 hour after phe-load	13.15 ( $\pm 4.91$ )	9.14 ( $\pm 5.17$ )	4.73 ( $\pm 2.60$ )	4.13 ( $\pm 2.13$ )
2 hours after phe-load	14.73 ( $\pm 4.77$ )	7.10 ( $\pm 5.02$ )	3.87 ( $\pm 1.91$ )	2.66 ( $\pm 1.49$ )
4 hours after phe-load	13.39 ( $\pm 4.85$ )	3.63 ( $\pm 4.59$ )	2.61 ( $\pm 1.36$ )	1.24 ( $\pm 0.62$ )

DRD = dopa-responsive dystonia; F-ITD = focal idiopathic torsion dystonia; G-ITD = generalized idiopathic torsion dystonia.

clear response to L-dopa. All other patients (F-ITD and G-ITD) included failed to show improvement on L-dopa treatment.

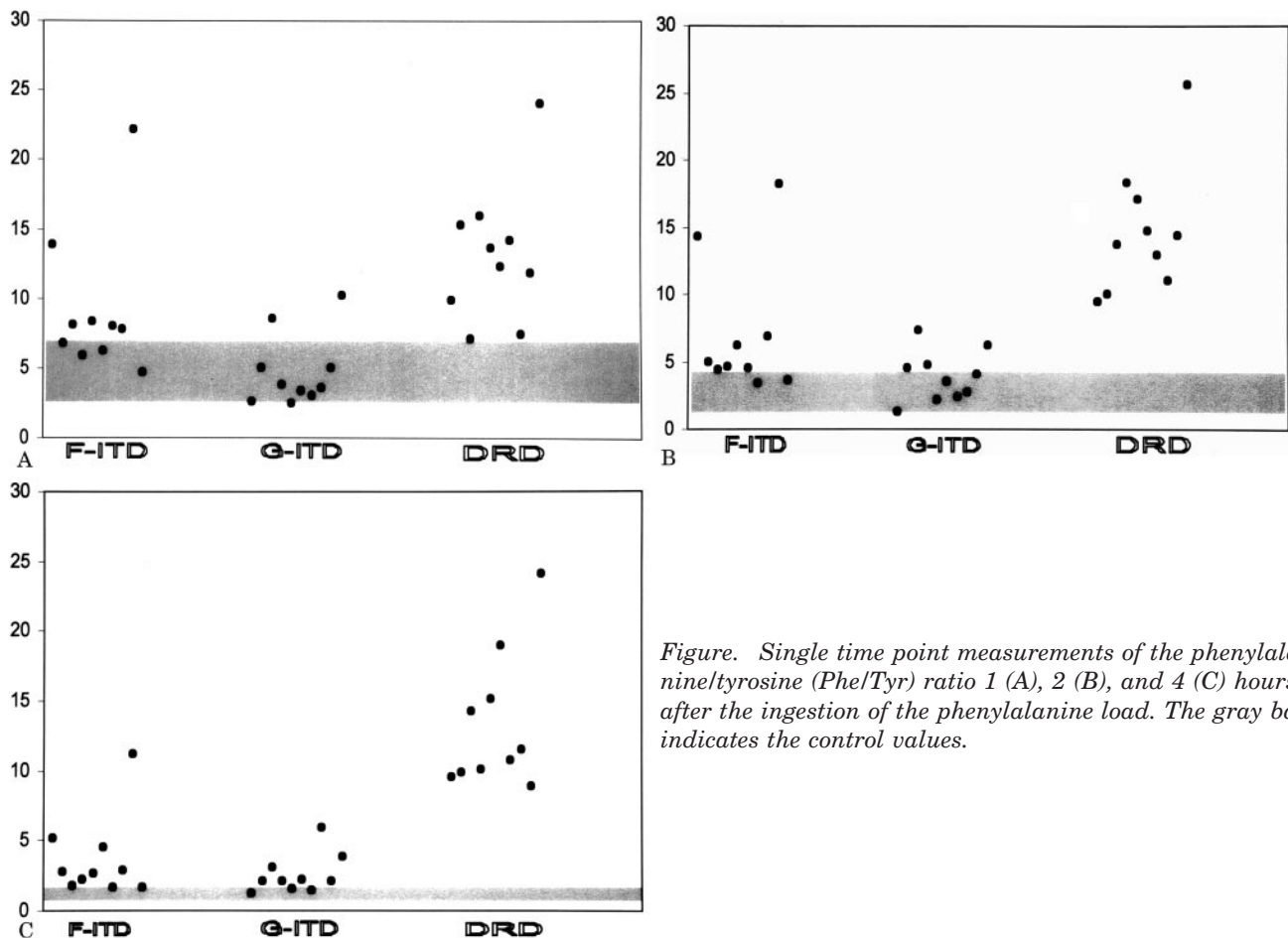
**Oral phenylalanine load.** The Phe-L was undertaken as previously described.<sup>7</sup> Blood samples were drawn into heparin tubes before the phenylalanine load and at 1, 2, and 4 hours postloading. Plasma was separated immediately, frozen on dry ice, and stored at  $-70^{\circ}\text{C}$ . Phenylalanine and tyrosine were measured using standard high pressure liquid chromatography methods as previously described.<sup>9</sup>

**Data analysis.** Values of phenylalanine, tyrosine, and the phenylalanine/tyrosine (Phe/Tyr) ratio were compared between patients with DRD and patients with F-ITD or G-ITD using the Mann-Whitney *U* test.

**Results.** The mean Phe/Tyr values before the Phe-L as well as 1, 2, and 4 hours afterwards are listed in the table. Single point values for 1, 2, and 4 hours are shown in the figure, A through C. The Phe/Tyr values between the DRD group and the F-ITD and G-ITD groups were different at all time points (DRD vs F-ITD, 1 hour after Phe-L:  $p = 0.041$ ; 2 hours: 0.005; 4 hours: 0.008; DRD vs G-ITD, 1 hour after Phe-L:  $p = 0.0007$ ; 2 hours:  $p = 0.0002$ ; 4 hours:  $p = 0.0002$ ). However, as shown in the figure, A through C,

there is considerable overlap 1 hour after the Phe-L for the single patient analysis but hardly any overlap 4 hours after the Phe-L. Using a cut-off value for the Phe/Tyr ratio of 7.5, no patient with G-ITD and only one patient with F-ITD, but all patients with DRD, had pathologic values.

**Discussion.** We investigated the possible relevance of the Phe-L in the differential diagnosis of DRD. All patients with DRD were readily identified, reflecting a very high sensitivity of the Phe-L (100%). Furthermore, only 1 of the 20 patients with ITD tested had a Phe/Tyr ratio similar to patients with DRD 4 hours after the Phe-L, reflecting the very high specificity of this test (95%). This indicates that a possible introduction of the Phe-L into clinical practice may lead to a reliable identification of patients with DRD, but that unnecessary treatment with L-dopa cannot be avoided in all cases. There



**Figure.** Single time point measurements of the phenylalanine/tyrosine (Phe/Tyr) ratio 1 (A), 2 (B), and 4 (C) hours after the ingestion of the phenylalanine load. The gray bar indicates the control values.

was no overlap between the Phe/Tyr ratios of any of the patients with DRD and the controls at 2 and 4 hours after the phenylalanine load.

The patient with F-ITD with the unusually high Phe/Tyr ratio was followed up by lumbar puncture, genetic analysis of the phenylalanine hydroxylase gene (*PAH*), and an 8-week trial of L-dopa (125 mg three times a day). Biopterin, neopterin, and catecholamine metabolite values in the CSF were normal, no mutation was detected in the *PAH*, and her dystonia failed to improve on L-dopa treatment. Her abnormal Phe/Tyr ratio may reflect a heterozygote carrier state of one of the other BH4 or phenylalanine metabolizing enzymes; she should accordingly be classified as false positive.

Previously, the analysis of the Phe/Tyr ratio was undertaken 1, 2, 4, and 6 hours after the Phe-L.<sup>7</sup> However, our data indicate that single point analysis 4 hours after the Phe-L is sufficient. This would facilitate the introduction of the Phe-L into routine clinical practice. Our data also indicate that routine BH4 measurement after the Phe-L is not necessary to achieve high sensitivity and specificity.<sup>10</sup> This further facilitates clinical testing because BH4 measurement is technically demanding and is only undertaken in a limited number of laboratories. It should be noted that at least one other group has not found the Phe-L to be absolutely specific and sensi-

tive for DRD.<sup>10</sup> If our results are confirmed by others, the introduction into routine clinical practice of the Phe-L as an easy to use, comparatively cheap, and quick diagnostic test for DRD might be helpful to ensure early treatment of patients with DRD.

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# Activated CD8+ T cells in secondary progressive MS secrete lymphotoxin

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**Abstract**—The authors compared the functional activation state and cytokine secretion profile of CD8+ T cells in patients with relapsing-remitting and secondary progressive (SP) MS to those in normal controls. In addition, they examined cytokine secretion in relationship to single nucleotide polymorphism (SNP) analysis of cytokine genes. A significant increase in lymphotoxin secretion from anti-CD3-stimulated CD8+ T cells was observed in patients with SPMS as compared to normal controls. The authors found no significant differences in SNP frequency or in secretion of other cytokines.

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MS is an inflammatory disease of the CNS white matter marked by early infiltration of both CD4+ and CD8+ T cells into the characteristic plaque that is the pathologic hallmark of the disease<sup>1</sup> and by activated, costimulation-independent, myelin-reactive CD4+ T cells in the peripheral blood.<sup>2</sup> Although CD8+ T cells

are present at less than half the absolute frequency of CD4+ T cells in normal peripheral blood, they outnumber CD4+ T cells in a perivascular distribution and at the active edge of chronic CNS plaques.<sup>1</sup> In contrast to relapsing-remitting MS (RRMS), secondary progressive MS (SPMS) is relatively unre-

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