RESEARCH REPORT

Urinary Neopterin and Phenylalanine Loading Test as Tools for the Biochemical Diagnosis of Segawa Disease

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Abstract *Background*. The diagnosis of autosomal dominant GTP-cyclohydrolase deficiency relies on the examination of the *GCH1* gene and/or pterins and neurotransmitters in CSF. The aim of the study was to assess the diagnostic value, if any, of pterins in urine and blood phenylalanine (Phe) and tyrosine (Tyr) under oral Phe loading test.

Methods. We report on two new pedigrees with four symptomatic and four asymptomatic carriers whose pattern of urinary pterins and blood Phe/Tyr ratio under oral Phe loading pointed to GTP-cyclohydrolase deficiency. The study was then extended to 3 further patients and 90 controls. The diagnostic specificity and sensitivity of these metabolic markers were analysed by backwards logistic analysis.

Results. Two genetic alterations segregated alternatively in Family 1 (c.631-632 del AT and c.671A > G), while exon 1 deletion was transmitted along three generations in Family 2. Neopterin and biopterin concentrations in urine clustered differently in controls under and over the age of 15. Therefore patients and controls were sub grouped according to this age. Neopterin was significantly reduced in GCH1 mutated subjects younger than 15, and both neopterin and biopterin in those older than 15. Moreover, the Phe/Tyr ratios at the second and third hour were both significantly higher in patients than in controls. Backwards logistic regression demonstrated the high diagnostic sensitivity and specificity of combined values of neopterin concentration and Phe/Tyr ratio at the second hour.

Conclusions. Pterins in urine and Phe loading test are non-invasive and reliable tools for the biochemical diagnosis of GTP-cyclohydrolase deficiency.

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Introduction

Autosomal dominant (AD) GTP-cyclohydrolase (GTP-CH EC 3.5.4.16) deficiency (DYT5a; MIM # 600225) presents with a wide spectrum of Dopa-responsive movement disorders (DRD) and a variable and sex-conditioned penetrance (Segawa et al. 2003; Segawa 2009). *GCH1* gene alterations are found in about 60% of patients (Segawa 2009), while in almost all cases neopterin and biopterin are low in CSF (Fink et al. 1988; Furukawa et al. 1993; LeWitt et al. 1986).

Pterins in urine, even though rarely assessed, are generally considered normal (Furukawa et al. 1998). We report on two new families with genetically confirmed DYT5a whose pattern of urinary pterins coupled with phenylalanine (Phe)/ tyrosine (Tyr) ratio under oral Phe loading pointed to GTP-GH deficiency. On this perspective the biochemical hallmarks

of the disease were reviewed and the potential diagnostic value of Phe loading test and pterins in urine was explored.

Patients

Family 1. The proband, a 12-year-old girl, was born after a normal pregnancy and delivery from Italian unrelated parents. Mother and a 9-year-old brother were normal. Her 36-year-old father had been suffering since adolescence from gait fatigability and foot rigidity that increased in the evening period. The patient's early psychomotor development was considered normal. At the age of 3 years, she suffered from atonic fits and was treated with antiepileptic medicaments. When 8 years old, she was again examined because of learning difficulties that were ascribed to a mild mental retardation. Starting from the age of 9 years, she complained of fatigability and instable gait emerging during the evening period. On examination, at the age of 11, she exhibited generalised choreoathetosis, which was exacerbated by exercise and in the late afternoon and evening when she was no longer capable of walking unsupported. She was mildly mentally retarded (WISC-R IQ 51), depressed and anxious. On neurological examination her father showed a mild foot dystonia with diurnal fluctuation.

Molecular analysis of the *GCH1* gene (see below for the methods) disclosed a frameshift mutation in exon 6 (c.631-632 del AT, p. M211fs) in the girl and in her father (case 1a and b, respectively, Table 1). Unexpectedly, a different point mutation was found in the patient's mother and brother (c.671A>G, p.K224R) (case 1c and 1d, Table 1).

L-Dopa/Carbidopa treatment (up to 8/2 mg/kg bw/day) resulted in a dramatic improvement in the father and in the proband. At the same time her WISC-R IQ improved to 63.

Family 2. A 7.5-year-old girl of Swedish and Italian descent presented with a progressive long-lasting severe gait disorder with diurnal fluctuation. She was the first offspring of two unrelated parents. The 32-year-old mother had been complaining during childhood of remarkable fatigability that disappeared in the following years. The proband was born after a normal pregnancy and delivery. She was able to walk unsupported at the age of 13 months when the parents first noticed fatigability and protracted uncertainty in the gait. At the age of 3, a mild paraparesis was diagnosed. During the following few years paraparesis worsened and a diurnal fluctuation of the symptoms became manifest. On examination, at the age of 7, she showed normal mental development (WISC-III IQ 115), generalised hypo- and bradykinesia, dystonic paraparetic gait with extra rotation of the hip and feet drop in the propulsive phase of gait and masked face with rigid fixed posture of head and neck. Exon 1 deletion in the *GCH1* gene was detected in the proband, her brother, mother and unaffected 58-year-old maternal grandmother of

Swedish origin (respectively cases 2a-2d in Table 1) (see below for the methods).

The only neurological sign detected in the mother was an exaggerated dorsal flexion of the big toe in the oscillatory phase of the gait.

Under L-Dopa/Carbidopa treatment (2/0.5 mg/kg bw/day), the girl experienced a marked improvement of both hypo-bradykinesia and lower limb dystonia.

Methods

Genomic analysis included the following: exon and intron–exon boundaries sequencing (Bandmann et al. 1996), screening for intra-gene deletions or duplications of the alleles negative to sequencing analysis (Zirn et al. 2008) (SALSA MLPA KIT, MRC-Holland, Amsterdam, The Netherlands) (Schouten et al. 2002) and retesting of the patients positive to MLPA analysis with Real-Time PCR (SYBR Green dye chemistries).

Informed consent was obtained from all subjects examined and (if minor) their parents.

5-Hydroxyindoleacetic acid [5-HIAA], homovanillic acid [HVA], and 3-methoxy-4-hydroxyphnylglycol were assessed in CSF by high-performance liquid chromatography with electrochemical detection. Neopterin and biopterin were determined in the first morning urine sample and CSF according to the method published elsewhere (Antonozzi et al. 1988). Phe and Tyr were measured by ESI-MS/MS (Chace et al. 1993) in dried blood spots at baseline and 1, 2, 3, 4, 5 and 6 h after oral Phe loading (100 mg/kg body weight) (Hyland et al. 1997).

To evaluate specificity and sensitivity of these tests for the diagnosis of Segawa disease, the study was extended to 3 previously diagnosed patients (Table 1, cases 3–5) and 90 subjects with primary and secondary movement disorders not due to defects of biogenic amine metabolism who acted as control samples. In the whole, pterins in urine were assessed in 101 subjects, while 9 subjects with GCH1 mutations and 39 controls also underwent Phe loading test. No subject among patient and control population showed clinical and/or haematological features of concurrent infection disease when the urine sample was collected.

Statistical analysis. Quantitative data are presented as means $\pm$ standard deviations, and were analyzed by Mann–Whitney U test to assess differences between groups within each age class. Stepwise logistic regression analysis (backwards selection) was performed to select the variables associated with the diagnosis, separately in the two age groups. Potential diagnostic factors were biopterin, neopterin, Phe peak, Phe/Tyr at the second and third hour after Phe loading. The estimated Receiver Operating Characteristic (ROC) curve was then plotted for the selected model

Table 1 Genotype and biochemical phenotype in CGH1 mutated subjects enclosed in the study (see text for clinical details)

ID	Genotype	Sex	Age (years)	CSF HVA[a] (r.v.)[b]	CSF 5-HIAA[a] (r.v.)[b]	CSF NEO[a] (r.v.)[b]	CSF BIO[a] (r.v.)[b]	Urine NEO[a] (r.v.)[c]	Urine BIO[a] (r.v.)[c]	Ref.
1a	c.631-632 del AT [p.M211fs]	F	11	229 (148–434)	143 (68–115)	**7.7** (9.1–20.1)	11.8 (10.1–29.9)	**0.27** (0.30–1.84)	1.21 (0.53–2.05)	Pedigree 1: proband
1b	c.631-632 del AT [p.M211fs]	M	36					0.23 (0.19–0.91)	0.69 (0.28–0.86)	Pedigree 1: father of 1a
1c	c.671A>G [p.K224R]	M	8					**0.22** (0.30–1.84)	0.65 (0.53–2.05)	Pedigree 1: brother of 1a
1d	c.671A>G [p.K224R]	F	34					0.41 (0.19–0.91)	0.36 (0.28–0.86)	Pedigree 1: mother of 1a
2a	Exon 1 deletion	F	7	188 (137–582)	74 (68–220)	**5.4** (9.1–20.1)	11.1 (10.1–29.9)	**0.23** (0.30–1.84)	**0.42** (0.53–2.05)	Pedigree 2: proband
2b	Idem	F	36					**0.13** (0.19–0.91)	**0.16** (0.28–0.86)	Pedigree 2: mother of 2a
2c	Idem	M	5					**0.25** (0.30–1.84)	0.60 (0.53–2.05)	Pedigree 2: brother of 2a
2d	Idem	F	58					**0.13** (0.19–0.91)	**0.16** (0.28–0.86)	Pedigree 2: maternal grandmother 2a
3	c.68 C>T [p.P23L]	M	25	**81** (98–450)	45 (45–135)	**5.1** (9.1–20.1)	10.5 (10.1–29.9)	**0.11** (0.19–0.91)	**0.19** (0.28–0.86)	Leuzzi, unpublished case
4	c.671A>G [p.K224R]	M	17	**80** (98–450)	**42** (45–135)	**8.7** (9.1–20.1)	16.9 (10.1–29.9)	**0.16** (0.30–1.84)	**0.47** (0.53–2.05)	Leuzzi et al. 2002
5	c.262 C>T [p.R88W]	F	45					**0.14** (0.19–0.91)	0.4 (0.28–0.86)	Leuzzi, unpublished case

HVA homovanillic acid, *5-HIAA* 5-hydroxyndoleacetic acid, *NEO* neopterin, *BIO* biopterin, *BH4* tetrahydrobiopterin

[a] Pathological values are in bold

[b] Age-related reference values (r.v.) (nmol/l)

[c] Age-related reference values (r.v) (2.5–98 percentile of control values; mmol/mol creatinine)

and for the models including each of the selected variables, separately considered. The areas under the ROC curves were calculated as an overall measure of diagnostic efficiency of the selected criterion. Sensitivity (Se), specificity (Sp) and positive and negative predictive values (PPV and NPV, respectively) were computed. Finally, a curve representing the diagnostic test based on the selected model was determined.

All the statistical analyses were performed using STATA Statistical Software (Release 8.0).

Results

Table 1 shows GCH1 genotype and biochemical phenotype of patients and asymptomatic carriers from the two families. Neopterin was low in CSF of both propositi (1a, 2a). The pattern of pterin excretion in urine (Table 1 cases 1a-2d) and Phe/Tyr ratio under oral Phe loading in symptomatic (4) and pre-symptomatic (4) carriers from the two families pointed both to GTP-GH deficiency.

The preliminarily exploration of the levels of neopterin and biopterin in urine from the controls showed they clustered differently before and over the age of 15 (Fig. 2a and b). Mean values of biopterin and neopterin in the "15–17 years" group were significantly different from those observed in the "0–14 years" group ($p = 0.0003$ and $p = 0.0137$, respectively), while they did not differ from those observed in the "18–74 years" group ($p = 0.8121$ and $p = 0.7979$, respectively). At the ratio Phe/Tyr under Phe loading test at second hour, no significant differences were observed in both comparisons ($p = 0.4857$ and $p = 0.1615$, respectively (Fig. 1c shows the distribution of Phe/Tyr at second hour). Therefore, for the following statistical analysis, GCH1 mutated subjects and controls were all grouped according to their age (pterin analysis: $59 < 15$ and $42 \geq 15$ years; Phe loading test: $23 < 15$, $25 \geq 15$ years).

Neopterin and biopterin concentrations (mmol/mol creatinine) in urine were, respectively, 0.66 ± 0.31 (range 0.22–1.84) and 0.99 ± 0.39 (range 0.47–2.54) in controls younger than 15, and 0.44 ± 0.20 (range 0.13–0.94) and 0.60 ± 0.16 (range 0.18-0.89) in those aged >15. In GCH1 mutated subjects, they were respectively 0.24 ± 0.02 (range 0.22–0.27) and 0.72 ± 0.34 (range 0.42–1.21), and 0.22 ± 0.11 (0.11-0.41) and 0.41 ± 0.19 (range 0.16-0.69) (GCH1 mutated subjects vs controls: <15 years neopterin $p = 0.0013$, biopterin $p = 0.1066$; ≥ 15 years neopterin $p = 0.0053$, biopterin $p = 0.0216$). Phe/Tyr ratio at seconnd and third hour after the loading of Phe were both significantly higher in patients than in controls (< 15 years: 5.59 ± 3.34 vs 1.91 ± 0.62, $p = 0.0285$; and 4.48 ± 3.88 vs 1.43 ± 0.47, $p = 0.0621$, respectively; ≥ 15 years:
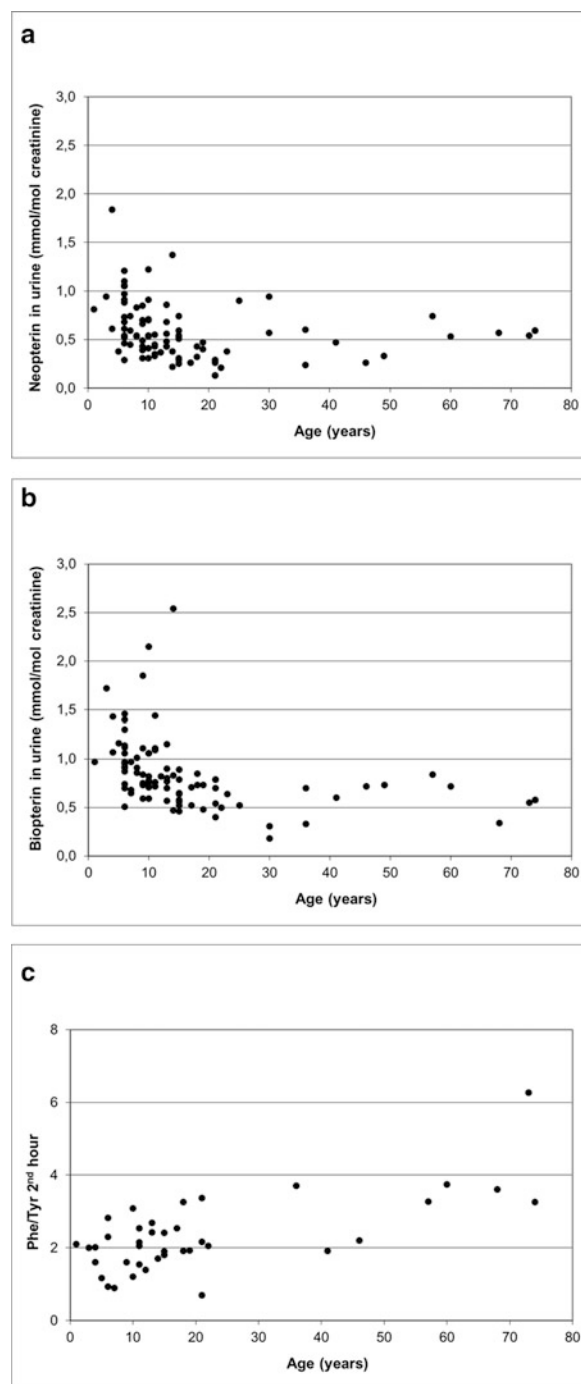
Fig. 1 Scatter plot of neopterin concentrations in urine vs age in controls ($n = 90$; $r = -0.19$; $p = 0.0748$) (a), biopterin concentrations in urine vs age in controls ($n = 90$; $r = -0.37$; $p = 0.0003$) (b) and Phe/Tyr ratio 2 h after Phe loading vs age in controls (c) ($n = 39$; $r = 0.68$; $p < 0.0001$)

7.27 ± 2.44 vs 2.73 ± 1.18, $p = 0.0006$; and 5.69 ± 2.36 vs 2.07 ± 0.84, p $= 0.0007$, respectively).

Backwards logistic regression identified Phe/Tyr at second hour and urine neopterin as significantly associated to GCH1 gene alteration. The combined criterion appeared

Table 2 Results of the logistic regression analyses assessing the diagnostic value of Phe/Tyr ratio 2 h after Phe loading and neopterin concentration in urine

Area under the ROC curve	Phe/Tyr 2nd hour		Neopterin[o]		Phe/Tyr 2nd hour + Neopterin[o]	
	Age					
	<15 years (n = 23)	≥15 years (n = 25)	<15 years (n = 59)	≥15 years (n = 42)	<15 years (n = 23)	≥15 years (n = 25)
Se %	66.7	83.3	75.0	42.9	100.0	100.0
Sp %	100.0	94.7	98.2	94.3	100.0	100.0
PPV %	100.0	83.3	75.0	60.0	100.0	100.0
NPV %	95.2	94.7	98.2	89.2	100.0	100.0
Correctly classified %	95.7	92.0	96.6	85.7	100.0	100.0
Cut-off [a]	3.84	5.24	0.26	0.15	See Fig. 2a	See Fig. 2b
Logistic regression coefficients					See 'Results' and Fig. 2a	See 'Results' and Fig. 2b
Constant	−6.90	−7.59	11.48	1.48		
Independent variable	1.79	1.45	−44.12	−10.09		
p for the coefficient of the selected independent variable	0.145	0.022	0.053	0.022		

Se sensitivity, *Sp* specificity, *PPV* positive predictive values, *NPV* negative predictive values

[o] mmol/mol creatinine

[a] Cut-off values were obtained by logistic regression coefficients

to perform better in terms of specificity and sensitivity than the criteria based on Phe/Tyr at second hour and neopterin separately considered (Table 2). Considering both Phe/Tyr at second hour and neopterin values, the decision rule can be based on the following analytical expressions, obtained by the multivariate logistic regression (ROC analysis). The test is positive if:

"-27.47 - 464.38 * [neopterin] + 63.93 * Phe/Tyr 2 h > 0" for subjects younger than 15 years (Fig. 2a), and "-106.91 - 456.85 * [neopterin] + 53.48 * Phe/Tyr2h > 0" for subjects older than 15 years (Fig. 2b), where [neopterin] is the concentration of neopterin in urine (expressed in mmol/mol creatinine) and Phe/Tyr2h is the ratio between Phe and Tyr concentrations in dried blood spot (each expressed in μmol/l of blood) 2 h after Phe loading.

Discussion

The *GCH1* gene mutation is the most common cause of early onset DRD (Hagenah et al. 2005; Clot et al. 2009), involving about 85% of all patients with DRD (Furukawa 2004). More than 140 alterations have been so far reported on the GCH1 gene (www.hgmd.cf.ac.uk, accessed 17 November 2011). Two pathogenetic mutations occurred independently in family 1. p.M211fs was detected in the proband, who presented moderate mental retardation years before developing generalized choreoathetosis with diurnal fluctuation. Both symptoms, and remarkably movement disorders, improved with L-Dopa/Carbidopa therapy. A mild dystonic phenotype was detected in her father. p.M211fs has been so far associated with a typical DRD presentation (Hagenah et al. 2005; Clot et al. 2009; Trender-Gerhard et al. 2009). Different to what happens in the recessive disorders of biogenic amine metabolism (Leuzzi et al. 2010), mental retardation is not part of DYT5a phenotype. It has been reported in the context of a complex presentation due to a 2.3 Mb deletion on chromosome 14q21-22 (family ITD612 in Clot et al. 2009) and in a single patient with a biochemically confirmed diagnosis but no alteration on the *GCH1* gene (Nagata et al. 2007). Two other members of Family 1 carried p.K224R mutation and were clinically normal in spite of some biochemical alterations suggesting GTP-CH deficiency (see below). p.K224R has been so far reported in three pedigrees associated with athetoid cerebral palsy (family Hu in Bandmann et al. 1998), myoclonic dystonia (Leuzzi et al. 2002) and adult-onset gait dystonia, rigidity and bradykinesia (Patient D97 in Garavaglia et al. 2004). Since the first report (Furukawa et al. 2000), few pedigrees harbouring gross deletion of the *GCH1* gene have been reported (Klein et al. 2002; Hagenah et al. 2005; Ohta et al. 2006; Clot et al. 2009). Their phenotype overlaps with that of patients with less severe alterations.

Since the availability of an aetiological treatment, the diagnosis of DYT5a should be considered for each
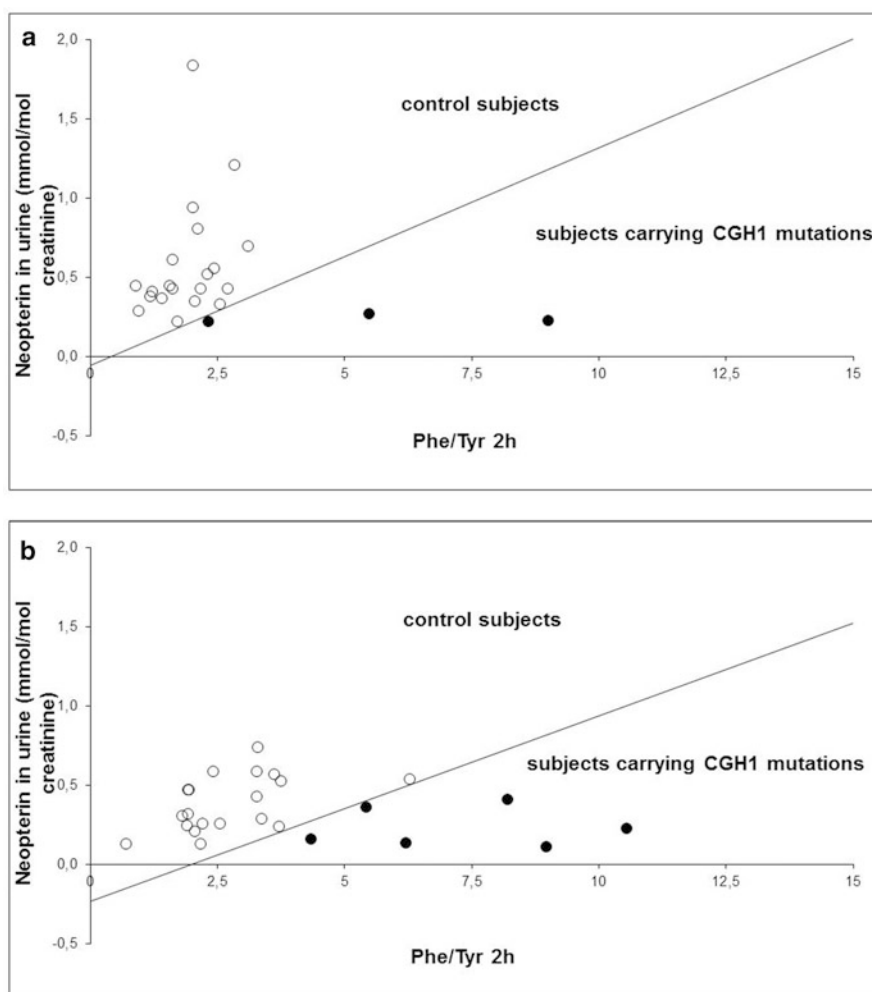
Fig. 2 Scatter plot of Phe/Tyr at second hour and neopterin values. The *white dots* represent controls (39) and the *black dots* patients (9) included in the ROC analysis. The curve divides the space into two areas, namely the area of control subjects above the curve and the area of subjects carrying GCH1 mutations below the curve. Each patient can be represented as a point in this space according to their values of neopterin and Phe/Tyr ratio 2 h after Phe loading: if the point lies in the area below the curve, the patient has a high probability of carrying a GCH1 mutations, whereas if they lie in the area above the curve, the patient has a high probability of not carrying a GCH1 mutation. Decision rule can be expressed by the following analytical expressions. The test is positive if "$-27.47 - 464.38 * [neopterin] + 63.93 * Phe/Tyr2h > 0$" for subjects younger than 15 years (**a**) and "$-106.91 - 456.85 * [neopterin] + 53.48 * Phe/Tyr2h > 0$" for subjects older than 15 years (**b**), where [neopterin] is the concentration of neopterin in urine (expressed in mmol/mol creatinine) and Phe/Tyr2h is the ratio between Phe and Tyr concentrations in dried blood spot (each expressed in μmol/l of blood) 2 h after Phe loading

patient presenting with an early onset dystonia-parkinsonism syndromes. Tables 1 and 3 summarize the *GCH1* genotype and biochemical phenotype in the few cases in which both were examined. CSF examination was performed on 23 patients (including ours) from 16 pedigrees. Neopterin was low in 21/23 cases, biopterin or tetrahydrobiopterin in 18/22, HVA in 11/15, and 5-HIAA in 8/15. As a group, the subjects carrying GCH1 mutations showed also a lower level of neopterin in urine in comparison with the controls.

GTP-CH is the limiting enzyme for the synthesis of tetrahydrobiopterin, which is the cofactor of phenylalanine, tyrosine and tryptophan hydroxylases (Blau et al. 2001).

Based on this, a Phe loading procedure has been proposed (Hyland et al. 1997) and different values of Phe/Tyr 4 hours after the loading have been suggested as a critical threshold for the diagnosis: 4.5 (Saunders-Pullman et al. 2000), 7.5 (Bandmann et al. 2003), 5.25 (Saunders-Pullman et al. 2004). In spite of high sensitivity and specificity of the test, false negatives (Saunders-Pullman et al. 2004) and positives (Bandmann et al. 2003) were reported. Recent data suggest that the ratio obtained at the second hour may be more sensitive in young patients with DRD (Opladen et al. 2010; Lopez-Laso et al. 2006). The test reliability improves if the peak value of blood Phe exceeds 600 μmol/L and the plasma and blood spot biopterin variations during the loading are

Table 3 CGH1 genotype and biochemical phenotype in patients with Segawa disease from the literature

ID[a]	Genotype	Sex	CSF HVA[b] (r.v.)[c]	CSF 5-HIAA[b] (r.v.)[c]	CSF NEO[b] (r.v.)[c]	CSF BIO/BH4[b] (r.v.)[c]	Ref.
6	c.218C>A [p.A73D]	M	**145** (364–870)	**151** (155–359)	7 (5–53)	**<2** (20–61)	Opladen et al. 2010 (pt 1)
7	c.623 + 3insT [IVS5 + 3insT]	F	**95** (217–507)	**62** (75–203)	**3** (7–27)	**3** (20–49)	Ibidem (pt 2)
8	GCH1mutation	F	414 (220–560)	174 (90–237)	**4** (7–27)	**6** (20–49)	Ibidem (pt 6)
9	Heterozygous deletion of all 6 exons	M	**112** (217–507)	**34** (75–203)	**2** (7–27)	**2**(20–49)	Ibidem (pt 7)
10	c.547G>A [p.E183K]	F			**18.8** (25 ± 5.0)	**9.7** (25 ± 5.0)	Ikeda et al. 2009
11	c.64_65delGGinsAACC [p.G21fsX66]	F	**48** (115–455)	**20** (51–204)	**6** (10–31)	**n.d.** (18–53)	von Mering et al. 2008
12	c.159delG [p.W53fs]	F			**3.1** (22.6 ± 1.5)	**3.8** (20.6 ± 1.4)	Furuya et al. 2006
13	c.265C>T [p.Q89X]	M	**268** (334–906)	236 (170–420)	**2** (8–43)	**8** (11–39)	Lopez-Laso et al. 2006
14a	c.618del15bp [p.V206fs]	F			**8.5** (22.1 ± 7.0)	**8.2** (26.4 ± 8.5)	Ohta et al. 2006
14b	c.626 + 1G>A [IVS5 + 1G>A]	F			**3.9** (22.1 ± 7.0)	**1.3** (26.4 ± 8.5)	Ibid(pt 4)
14c	Exons 3-4 deletion	F			**8.6** (22.1 ± 7.0)	**15.7** (26.4 ± 8.5)	Ibid(pt 5)
15a	c.411del 37 bp [p.F138fs]	M	**142** (395 ± 56)	**50** (126 ± 22)	**1.1** (> 20)	**5.4** (> 20)	Hahn et al. 2001
15b	c.411del 37 bp [p.F138fs]	M	**85** (268 ± 24)	**43** (108 ± 15)	**4.8** (> 20)	**6.8** (>20)	Ibid(III:3)
15c	c.411del 37 bp [p.F138fs]	F	**447** (528 ± 75)	161 (133 ± 14)	**18.5** (> 20)	27.8 (> 20)	Ibidem(IV:6)
16a	c.671A>G [p.K224R]	F			**5.9** (7–65)		Bandmann et al. 1996; (Family Hu, II-3)
16b	c.671A>G [p.K224R]	F	91 (72–656)	65 (58–222)	**5.9** (7–65)	**6.6** (BH4) (9–40)	Robinson et al. 1999; (Family Hu, II-4)
16c	c.671A>G [p.K224R]	M	152 (72–256)	49 (58–222)	**4.3** (7–65)	16 (BH4) (9–40)	Ibid(Family Hu, II-5)
17a	c.344-1G>A [IVS1-1G>A]				**4.4** (13.0–38.3)	**7.1** (9.6–21.2)	Furukawa et al. 1996; (pt 2)
17b	c.341C>A [p.S114X]				**8.9** (13.0–38.3).	**5.9** (9.6–21.2)	Ibid(pt 3)

HVA homovanillic acid, 5-HIAA 5-hydroxyndoleacetic acid, NEO neopterin; BIO biopterin, BH4 tetrahydrobiopterin

nd: not detectable

[a] Numeration follows that of Table 1

[b] Pathological values are in bold

[c] r.v.: reference values (nmol/l)

monitored (Opladen et al. 2010, Saunders-Pullman et al. 2004).

We have showed that the analysis of neopterin in first morning urine leads to a correct classification in 96.6% (age < 15aa) and 85.7% (age > = 15 years) of 101 analyzed samples (Table 3) and that the evaluation of both Phe/Tyr ratio at the second hour and neopterin in urine leads to the highest specificity and sensitivity in order to detect young and adult subjects with GCH1 alterations, even though it cannot discriminate between symptomatic and pre-asymptomatic carriers (Hyland et al. 1999).

A pattern of Phe/Tyr ratio mimicking that found in GTP-CH deficiency may be found in the carriers of Phenylalanine Hydroxylase gene mutations (Driscoll and Hsai 1956; Guldberg et al. 1998), who, however, have a normal excretion of pterins in urine.

Caution should be adopted in interpreting urine and serum neopterin values in the presence of concomitant conditions potentially leading to T cell–mediated immune response activation (Huber et al. 1984) (such as viral infections, autoimmune diseases, malignancies, pregnancy, etc) (Werner-Felmayer et al. 2002): they could stimulate neopterin synthesis, so masking a possible partial defect of GTP-CH.

In conclusion, even though conducted in a restricted number of subjects, our study suggests a new simple, reliable and non-invasive approach to the diagnosis of DYT5a. It could be part of the diagnostic workup for patients presenting with idiopathic movement disorders of unknown origin.

Synopsis

The authors propose a rapid, simple and non-invasive method for the diagnosis of AD-DRD and suggest it as part of the diagnostic workup for patients presenting with idiopathic movement disorders of unknown origin.

Author Roles

Vincenzo Leuzzi: Conception, Organization, Execution of Research project; Writing of the first draft, Review and Critique of Statistical Analysis.

Claudia Carducci: Conception, Organization, Execution of Research project, Biochemical Studies; Review and Critique of Statistical Analysis.

Flavia Chiarotti: Review and Critique of experimental design, Statistical Analysis,

Daniela D'Agnano: Organization, Execution of Research project; Clinical data collection and patient follow-up; Review and Critique of Statistical Analysis.

Italo Antonozzi: Conception, Organization, Execution of Research project; Writing of the first draft, Review and Critique of Statistical Analysis

Maria Teresa Giannini: Organization, Execution of Research project; Clinical data collection and patient follow-up. Review and Critique of Statistical Analysis.

Carla Carducci: Organization, Execution of Research project; Molecular analysis; Review and Critique of Statistical Analysis.

Guarantor

Vincenzo Leuzzi

Competing Interests

The authors have declared that no competing interests exist.

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Written Consent

A written consent of the legal substitute of the patients was obtained for genetic analysis.

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