

Sepiapterin reductase deficiency an autosomal recessive DOPA-responsive dystonia

Nico G. Abeling^{a,*}, Marinus Duran^a, Henk D. Bakker^b, Lida Stroomer^a, Beat Thöny^c,
Nenad Blau^e, Jan Booij^d, Bwee Tien Poll-The^c

^a Academic Medical Center, Laboratory Genetic Metabolic Diseases, University of Amsterdam, Amsterdam, The Netherlands

^b Department of Pediatrics, University of Amsterdam, Amsterdam, The Netherlands

^c Department of Pediatric Neurology Emma Children's Hospital, University of Amsterdam, Amsterdam, The Netherlands

^d Department of Nuclear Medicine, University of Amsterdam, Amsterdam, The Netherlands

^e Laboratory of Clinical Chemistry and Biochemistry, University Children's Hospital Zürich, Switzerland

Received 31 January 2006; received in revised form 20 March 2006; accepted 20 March 2006

Available online 2 May 2006

Abstract

The diagnosis of a 14-year-old girl with a new homoallelic mutation in the sepiapterin reductase (SR) gene is reported. Initially she presented at the age of 2 with hypotonia and mild cognitive developmental delay, and was diagnosed as having mild methylmalonic aciduria, which was recently identified as methylmalonylCoA racemase deficiency, a new defect in valine-isoleucine metabolism. After a 12-year progression of her neurologic condition, which had made her wheelchair-bound at the age of 6, dystonia with diurnal variation had become apparent. At the age of 14 this finding led to rapid diagnosis of SR deficiency. The diagnostic approach with CSF neurotransmitter and pterins analysis and combined phenylalanine/BH₄ loading test, and finally measurement of sepiapterin in CSF is illustrative for the diagnosis of SR deficiency. As in all other patients with this new defect, very low levels of homovanillic acid and 5-hydroxyindoleacetic acid and high levels of biopterin and sepiapterin in the CSF are the diagnostic hallmark. The girl improved dramatically on treatment with L-DOPA and 5-hydroxytryptophan. The initial diagnosis of methylmalonic aciduria may afterwards be considered to have not significantly contributed to her clinical condition and only has led to a long delay of the clinically relevant diagnosis of SR deficiency. Although the clinical condition of this recently recognized autosomal recessive defect in pterin metabolism is complex and many symptoms can occur in variable severity and time of onset, dystonia with diurnal variation is a characteristic finding, as shown in nearly all patients described so far. The rapid and favourable response on treatment with L-DOPA warrants the classification of SR deficiency as another autosomal recessive type of DOPA-responsive dystonia (DRD). This classification is important to improve the awareness of clinicians that more than one metabolic defect can underlie the phenotype of a DOPA-responsive dystonic disorder and that dystonia should always trigger a rapid diagnosis of the underlying neurotransmitter synthesis defect, in view of the excellent treatability of a DRD. © 2006 Elsevier Inc. All rights reserved.

Keywords: Sepiapterin reductase; Dystonia; DOPA-responsive; Pterins; Neurotransmitters

Introduction

DOPA-responsive dystonias (DRD) comprise a genetically heterogeneous group of movement disorders related to abnormal biogenic amine neurotransmitter metabolism.

Autosomal dominant DRD, first described by Segawa [1], is caused by mutations in the GTP cyclohydrolase I (GCH I) gene, and is generally characterized by childhood-onset DRD with diurnal fluctuation. GCH I deficiency leads to a decreased production of tetrahydrobiopterin (BH₄), the cofactor for a number of hydroxylases converting phenylalanine to tyrosine, tyrosine to DOPA, and tryptophan to 5-hydroxytryptophan (5-HTP), and for nitric oxide synthase [2], (Fig. 1A). Mutations in the tyrosine hydroxylase

* Corresponding author. Fax: +31 206962596.

E-mail address: n.g.abeling@amc.nl (N.G. Abeling).

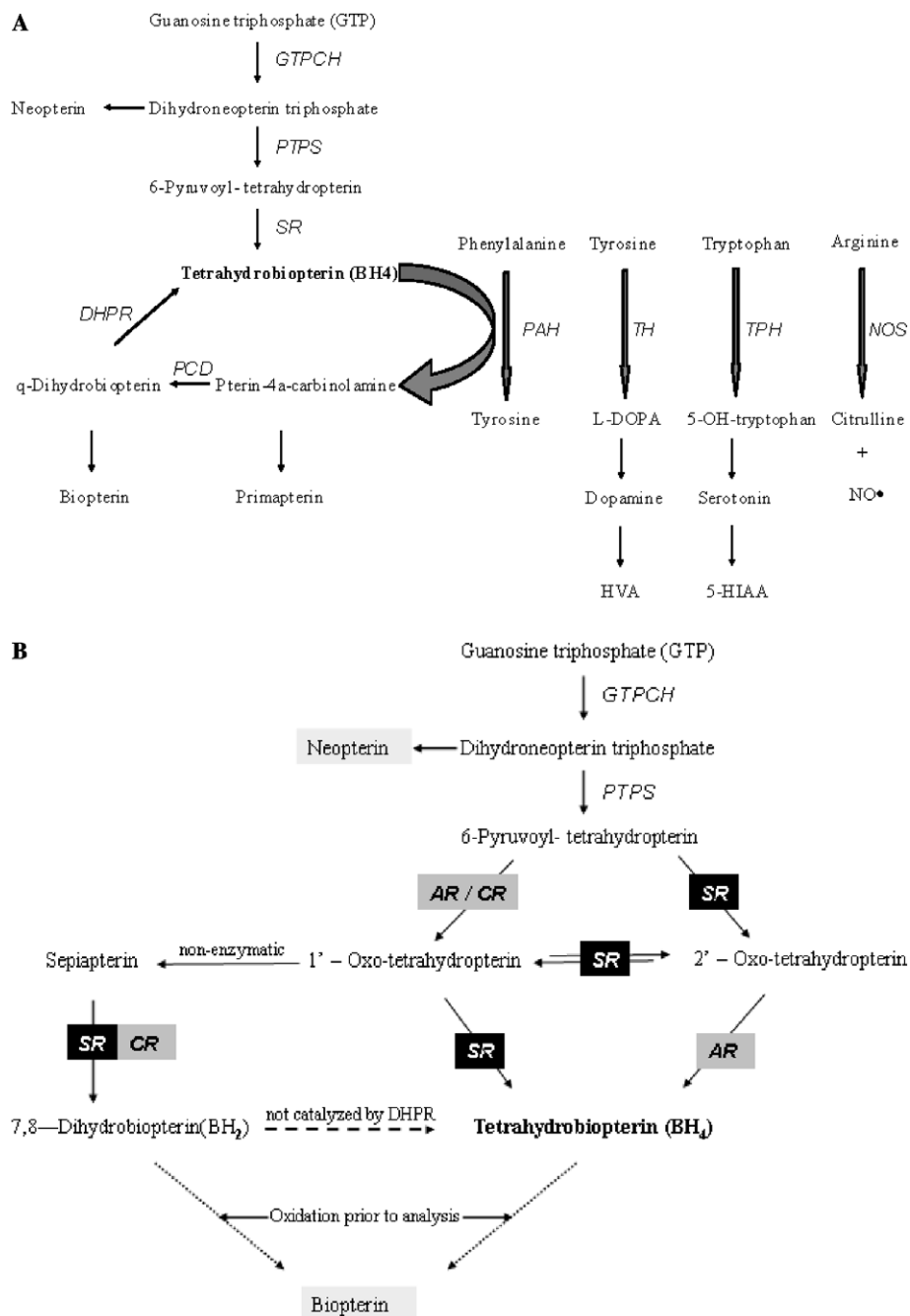


Fig. 1. (A) Metabolic pathways showing the role of sepiapterin reductase (SR) in the biosynthesis of BH₄ and the function of BH₄ in the aromatic amino acid hydroxylating and nitric oxide synthase (NOS) reactions. Enzymes: GTPCH, GTP cyclohydrolase; PTPS, 6-pyruvoyl-tetrahydropterin synthase; PCD, pterin-4a-carbinolamine dehydratase; DHPR, dihydropteridine reductase; PAH, phenylalanine hydroxylase; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase. Metabolites: HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; NO, nitric oxide. (B) Proposed (Bonafé [2]) alternative routes in brain in SR deficiency, showing the formation of a non-quinonoid form of BH₂, which is no substrate for DHPR, and the in vitro oxidation of both reduced biopterins as a sample pretreatment prior to analysis. CR, carbonyl reductase; AR, aldose reductase.

(TH) gene, resulting in a defective TH and hence a shortage of DOPA, cause autosomal recessive DRD [3].

Sepiapterin reductase (SR) deficiency is the most recently discovered inherited defect in the biosynthesis of BH₄. The major symptoms of the SR deficiency in the first three described patients were mental retardation, dystonia with diurnal fluctuations, axial hypotonia, and spasticity [2,4,5]. These patients responded favourably to treatment

with oral supplements of L-DOPA and 5-hydroxytryptophan in a combination with Carbidopa. Very recently seven additional SR-deficient patients in a genetic isolate from Malta were described [6], with clinical characteristics essentially comparable with those described in our patient and the three first cases. SR deficiency is considered to be an autosomal recessively inherited disease, the *SPR* gene maps to chromosome 2 p14-p12. The diagnosis of SR deficiency

relies on the finding of sepiapterin in CSF. Thus far this required a rather laborious technique.

A generalized defect in the biosynthesis of BH₄ usually results in severe hyperphenylalaninemia and a monoamine neurotransmitter deficiency, the exceptions being the autosomal dominant GCH I deficiency and SR deficiency. Therefore, these disorders cannot be detected by neonatal screening. The absence of hyperphenylalaninemia in SR deficiency has been explained by the existence of a peripheral dihydrofolate reductase driven salvage pathway for BH₄ synthesis, which is absent in the brain [2] (Fig. 1B). Here, we describe a girl with SR deficiency with a progressive neurologic condition, dominated by hypotonia and later on also dystonia, and an impressive response on L-DOPA and 5-HTP treatment. The diagnosis was strongly delayed by the initial detection of an other metabolic defect in this patient.

Patient history

The patient is a 16-year-old girl, born to consanguineous Dutch parents. She had initially been referred at 2 years of age because of delayed psychomotor development, with hypotonia. She was able to sit, albeit with a tendency to sag forward, but could not walk independently. Her motor condition was characterized as generalized paresis with mild spastic diplegia. There was no pyramidal syndrome. Sometimes myoclonic jerks were observed. Also she was drooling. Abnormal eye movements, particularly oculogyric crises, were not seen. In addition to her motor delay, also a mild cognitive delay was established, mainly manifested as dysarthric and slow speech with only two to three word sentences. Based on a Denver Developmental Screening Test a reasonable social and behavioral development was assessed. Metabolic screening of urine and plasma revealed a mild methylmalonic aciduria, which at that time could not be further classified despite extensive investigation. In CSF, a small amount of methylmalonic acid was detected. Treatment with a protein-restricted diet attenuated the methylmalonic aciduria, but a progressive neurologic deterioration was observed. At the age of 5 years, she had spastic paraparesis, mild saccadic eye movements, 'mask faces,'

abnormal movements of her limbs with flexion- and extension patterns when the neck was flexed, and had difficulty initiating speech. There were only mild cognitive problems and repeated brain MRIs were normal. The girl became wheelchair-bound at the age of 6. In the following years she gradually developed a mild limb and neck dystonia, worsening over the day and upon stressful environmental factors. Eventually, at the age of 14, she was virtually inactive and appeared exhausted at the end of the day, necessitating her to go and sleep at 8 p.m. This development triggered the investigation of aromatic neurotransmitter metabolites in CSF, which revealed a severe deficiency of aromatic neurotransmitters (Table 1). Hyperphenylalaninemia (HPA) had never been observed, but to test the peripheral availability of BH₄, an oral loading test with L-phenylalanine, followed by BH₄ as we published earlier [4], was performed, with a clearly abnormal result.

After establishment of the diagnosis, SPECT imaging was performed to evaluate the possible loss of striatal dopamine transporters or D₂ receptors.

Treatment and follow-up

Treatment was started with L-DOPA (2.5 mg/kg/day), carbidopa 0.9 mg/kg/day, and 5-hydroxytryptophan (0.75 mg/kg/day) and induced a rapid and spectacular clinical improvement with respect to strength, energy, and mood. The dose of L-DOPA had to be gradually decreased to 1.45 mg/kg/day in four divided doses because of oral dyskinesia and myoclonic movements of the hands. A slow but sustained further improvement was observed in the following months. After one year of treatment the patient was able to walk short distances (100 m) with support, perform complex functions like playing computer games; her speech had greatly improved, her face and eye pursuit movements had normalized, and she was more bright-tempered than before. She attends a school for motor disabled but cognitively normal children. Biochemically the treatment led to a gradual increase and finally normalization of the HVA and 5-HIAA levels in the CSF, indicating full restoration of the biogenic amine neurotransmitter synthesis, (Table 1).

Table 1

Biochemical findings in CSF of patient L.R. with sepiapterin reductase deficiency at the time of diagnosis, and during treatment with L-DOPA and 5-hydroxytryptophan

Metabolites (nmol/l)	Diagnosis	Reference range ^a	1 month treatment	5 month treatment	8.5 month treatment	14 month treatment
HVA	76	148–434	136	113	92	291
5-HIAA	5	68–115	42	40	21	92
MHPG	5	28–60	12	170	29	44
3-OMD	30	<50	n.a.	632	251	10
DOPA	17	<25	n.a.	n.a.	n.a.	n.a.
5-HTP	2	<10	n.a.	n.a.	n.a.	n.a.
Neopterin	26	9–20	22	6	25	n.a.
Biopterin	55	10–30	64	79	68	n.a.
Sepiapterin	11	n.d.	n.a.	n.a.	10	n.a.

Abbreviations: HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; MHPG, methoxy-hydroxy-phenylglycol; 3-OMD, 3-O-methylDOPA; DOPA, dihydroxyphenylalanine; 5-HTP, 5-hydroxytryptophan. n.d., not detected; n.a., not analyzed.

^a Ranges for age-matched controls.

Methods

Biochemical analyses

Biogenic amines, their precursors and metabolites were analyzed in urine, using reverse phase high performance liquid chromatography (HPLC) methods with electrochemical and fluorometric detection [7,8]. These compounds were measured in CSF using reverse-phase HPLC and gradient elution with phosphate buffers followed by amperometric detection, mainly according to Scheinin et al. [9]. Pterins in CSF were analyzed after iodine oxidation using reverse-phase HPLC and gradient elution with phosphate buffers followed by fluorometric detection, essentially according to Sawada et al. [10]. Sepiapterin in CSF was measured by HPLC without pretreatment, using a Jasco (Jasco Inc., Easton, MD 21601, USA) fluorescence detector set at 425/530 nm (excitation/emission), as an adaptation to the original method of Zorzi et al. [11].

Fibroblast studies

Cell culturing, neopterin, and biopterin production in fibroblasts after stimulation with cytokines for 24 h, and SR activity measurement in non-stimulated fibroblasts were performed as described previously [12].

DNA mutation analyses

Mutation analyses of the *SPR* gene were performed in DNA isolated from blood samples of the index patient and of both parents using primers described previously [2].

SPECT imaging

The availability of striatal D₂ receptors and dopamine transporters were studied with ¹²³I-iodobenzamide (IBZM) SPECT and ¹²³I-fluoropropyl (FP)-CIT, respectively, using a high-resolution multidetector neuro-SPECT system (Strichman Medical Equipment, 810X).

Results

Analysis of CSF at the age of 14 years (Table 1) revealed very low HVA and 5-HIAA, and normal DOPA, 5-hydroxytryptophan and 3-*O*-methyl-DOPA. Neopterin and biopterin were clearly elevated in CSF (Table 1), while urine pterins were normal (data not shown). A phenylalanine loading test caused plasma phenylalanine to rise steeply to ca. 800 μmol/L, followed by a rapid, BH₄-induced normalization (Fig. 2). Sepiapterin in CSF was clearly elevated (Table 1). The sensitivity of our HPLC detection system took away the need for extensive purification of the biological sample, thereby making the diagnostic approach for SR deficiency routinely available.

These findings are consistent with SR deficiency, though the elevated CSF biopterin might be misleading. As a result of alternative, aldose reductase (AR) and carbonyl reductase (CR)-driven pathways existing in the brain, a non-quinonoid form of dihydrobiopterin (BH₂) can be produced, but not reduced by dihydropteridine reductase (DHPR) to BH₄ (Fig. 1B). As both BH₂ and BH₄ are oxidized prior to analysis, the sum of both compounds is measured as total biopterin.

Because BH₂ is produced in elevated amounts, total biopterin is measured as elevated despite the deficiency of BH₄ [2]. In cytokine-stimulated fibroblasts biopterin was

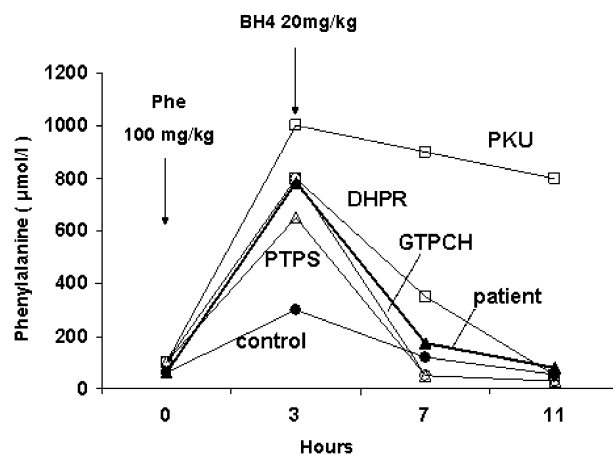


Fig. 2. Loading test with phenylalanine (100 mg/kg body weight) followed by BH₄ (20 mg/kg body weight), showing typical results for patients with defects in pterin metabolism and PKU-patients for comparison. The control person was not loaded with BH₄. GTPCH, GTP cyclohydrolase; PTPS, 6-pyruvoyl-tetrahydropterin synthase; DHPR, dihydropteridin reductase.

severely decreased (17.5 pmol/mg protein; controls 158–303), while neopterin was increased (199 pmol/mg protein; controls 18–98). This increase, in fibroblasts as well in CSF might be explained by inhibition of PTPS (Fig. 1) by sepiapterin or one of its degradation products [2]. SR activity in fibroblasts was severely decreased (18 μU/mg protein; controls 99–185). The establishment of a new, homoallelic mutation g.1437C>T (p.P163L) in exon 2 of the *SPR* gene in the patient, and heterozygosity in both parents completed the diagnosis.

The SPECT studies were performed before initiation of treatment and showed normal uptake of the radioligands in the regions of interest, indicating no loss of striatal dopamine D₂ receptors or transporters in this case of SR deficiency. This finding is in line with previous studies in DRD. It is also in keeping with the clinical observations of an excellent response to a low dose of L-DOPA.

Discussion

The diagnosis of sepiapterin reductase deficiency in this patient at the age of 14 years was delayed by an earlier diagnosis of an initially unclassified form of methylmalonic aciduria at the age of 2. At that time the hypotonia and delayed development were not considered to be suggestive of a neurotransmitter defect. The clinically relevant diagnosis was only made following the onset of dystonia with diurnal variation, when the patient was a teenager.

The low CSF levels of aromatic neurotransmitter metabolites in this patient were indicative of a defect in BH₄ synthesis. A sustained rise in plasma phenylalanine following an oral load with this amino acid supported this hypothesis, as also discussed by Van Hove et al. [13] in their paper on DRD. It should be emphasized that a major difference between GCH 1 deficiency and SR defi-

ciency lies in the CSF levels of biopterin. These levels are apparently elevated in SR deficiency and generally decreased in GCH 1 deficiency.

The establishment of SR deficiency fully explained the neurologic picture—including the normal brain MRI. At least in this case the hypothesis of a condition such as ‘central’ or ‘cerebral’ methylmalonic aciduria could safely be rejected. Meanwhile, the mild methylmalonic aciduria in this patient could be attributed to mutations in the methylmalonyl-CoA epimerase gene [14], a hitherto unknown inborn error of metabolism (to be published elsewhere). We have confirmed the fairly consistent neurologic picture in SR deficient patients [2,4,5], in which progressive (psycho)motor retardation, dystonia with diurnal fluctuation, early onset hypotonia and spasticity are the clinical hallmarks. Dystonia with diurnal fluctuation not only was the trigger for neurotransmitter analysis that rapidly led to establishment of the diagnosis, but also appeared to be a consistent and major element of the neurologic symptomatology in the previously reported SR-deficient patients. Dystonia was also described in five out of seven recently described Maltese patients with SR deficiency [6]. Also a dramatic, though incomplete response to L-DOPA was reported in all seven Maltese patients. In our patient, treatment with L-DOPA and 5-hydroxytryptophan was successful as well, and led to normalization of the CSF neurotransmitters after 14 months of treatment. Although a complete clinical normalization was not achieved, the response to treatment was impressive, in particular in view of the 12 years delay of the diagnosis. This raises the question to what extent the neurologic damage is reversible in this condition.

Variability in occurrence and severity of other symptoms of the condition, a.o. hypotonia, ataxia, tremors, spasticity, bulbar involvement, oculogyric crises, and cognitive impairment, is comparable with autosomal dominant GTPCH and tyrosine hydroxylase deficiency, which are both classified as forms of DOPA-responsive dystonia.

The homogeneous clinical phenotype with hypotonia followed by dystonia almost invariably as a major presenting symptom, and the favorable response on treatment with L-DOPA in all patients clearly classifies SR deficiency as another recessive form of DOPA-responsive dystonia in addition to the autosomal dominant GTPCH and the autosomal recessive tyrosine hydroxylase deficiency.

This classification is important to improve the awareness of clinicians that more than one metabolic defect can underly the phenotype of a DOPA-responsive dystonic disorder and that dystonia should always trigger a rapid diagnosis of the underlying neurotransmitter synthesis defect, in view of the excellent treatability of a DRD.

Acknowledgment

This work was supported in part by the Swiss National Science Foundation Grant No. 3100-066953.01 (to N.B.).

References

- [1] M. Segawa, A. Hosaka, F. Miyagawa, Y. Nomura, H. Imai, Hereditary progressive dystonia with marked diurnal fluctuation, *Adv. Neuro.* 14 (1976) 215–233.
- [2] L. Bonafé, B. Thöny, J.M. Penzien, B. Czarnecki, N. Blau, Mutations in the sepiapterin reductase gene cause a novel tetrahydrobiopterin-dependent monoamine neurotransmitter deficiency without hyperphenylalaninemia, *Am. J. Hum. Genet.* 69 (2001) 269–277.
- [3] A. Schiller, R.A. Wevers, G.C.H. Steenbergen, H.H. Blau, N. Jung, Long-term course of L-dopa-responsive dystonia caused by tyrosine hydroxylase deficiency, *Neurology* 63 (2004) 1524–1526.
- [4] N. Blau, L. Bonafé, B. Thöny, Tetrahydrobiopterin deficiencies without hyperphenylalaninemia: diagnosis and genetics of DOPA-responsive dystonia and sepiapterin reductase deficiency, *Mol. Genet. Metab.* 74 (2001) 172–185.
- [5] L. Elzaouk, H. Osmani, A. Romstad, J. Friedman, M. Maccolin, B. Thöny, N. Blau, Sepiapterin reductase deficiency: molecular analysis in a new case presenting with neurotransmitter deficiency without hyperphenylalaninemia, in: S. Milstien, G. Kapatios, R.A. Levine, B. Shane (Eds.), *Chemistry and Biology of Pteridines and Folates*, Kluwer Academic Publishers, Norwell, 2002, pp. 277–284.
- [6] B.G. Neville, R. Parascandalo, R. Farrugia, A. Felice, Sepiapterin reductase deficiency: a congenital dopa-responsive motor and cognitive disorder, *Brain* 128 (2005) 2291–2296.
- [7] N.G. Abeling, A.H. van Gennip, H. Overmars, P.A. Voute, Simultaneous determination of catecholamines and metanephrines in urine by HPLC with fluorometric detection, *Clin. Chim. Acta* 137 (1984) 211–226.
- [8] A.E. Stroomer, H. Overmars, N.G. Abeling, A.H. Van Gennip, Simultaneous determination of acidic 3,4-dihydroxy-phenylalanine metabolites and 5-hydroxy-indole-3-acetic acid in urine by high-performance liquid chromatography, *Clin. Chem.* 36 (10) (1990) 1834–1837.
- [9] M. Scheinin, W.H. Chang, K.L. Kirk, M. Linnoila, Simultaneous determination of 3-methoxy-4-hydroxyphenylglycol, 5-hydroxy-indoleacetic acid and homovanillic acid in cerebrospinal fluid with high-performance liquid chromatography using electrochemical detection, *Anal. Biochem.* 131 (1983) 246–253.
- [10] Y. Sawada, H. Shintaku, G. Isshiki, Neopterin and biopterin concentrations in cerebrospinal fluid in controls less than 1 year old, *Brain Dev.* 21 (4) (1999) 264–267.
- [11] G. Zorzi, U. Redweik, H. Trippe, J.M. Penzien, B. Thöny, N. Blau, Detection of sepiapterin in CSF of patients with sepiapterin reductase deficiency, *Mol. Genet. Metab.* 75 (2002) 174–177.
- [12] L. Bonafé, B. Thöny, W. Leimbacher, L. Kierat, N. Blau, Diagnosis of DOPA-responsive dystonia and other tetrahydrobiopterin disorders by the study of biopterin metabolism in fibroblasts, *Clin. Chem.* 47 (2001) 477–485.
- [13] J.L.K. Van Hove, J. Steyaert, G. Matthijs, E. Legius, P. Theys, R. Wevers, A. Romstad, L.B. Møller, K. Hedrich, D. Goriounov, N. Blau, C. Klein, P. Casaer, Expanded motor and psychiatric phenotype in autosomal dominant Segawa syndrome due to GTP cyclohydrolase deficiency, *J. Neurol. Neurosurg. Psychiatry* 77 (2006) 18–23.
- [14] T.A. Bobik, M.E. Rasche, Identification of the human methylmalonyl-CoA racemase gene based on the analysis of prokaryotic gene arrangements, *J. Biol. Chem.* 276 (2001) 37194–37198.