平成23年度厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

「小児神経伝達物質病の診断基準の作成と新しい治療法の開発に関する研究」

唾液中メラトニン濃度測定によるAADC欠損症のスクリーニング検査の可能性(2)

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研究要旨

重症心身障害児(者)（以下重症児（者））の中には、眼球上転発作やジストニアを示す例がありAADC欠損症が疑われる症例が存在する。しかし診断に必要な髄液採取は実施困難な場合が多く、重症児（者）でも抵抗なく実施可能な簡便なスクリーニング検査が望まれる。我々は、重症児（者）13例,正常対照5例、AADC欠損症患者3例に対して唾液中メラトニン濃度測定を行った。夜間唾液中メラトニン濃度はAADC欠損症群(4.8±3.5 pg/ml)では正常対照群(17.3±3.2 pg/ml)や重症児(者)群(31.4±16.1 pg/ml)より有意に低値だった。AADC欠損症をスクリーニングする方法として夜間唾液中メラトニン濃度測定は有効であると考えた。

A．研究目的

AADC欠損症の診断には髄液検査（HVA,5HIAAなどモノアミンやセロトニンの代謝産物の濃度測定）が必須である。しかし未診断のまま学齢期や成人となった重症児（者）や軽症例では診断に対するニーズが低く、痛みを伴う髄液採取を行うことは難しい。

そのため、より簡便で侵襲の少ないスクリーニングとして唾液中メラトニン濃度測定が使用できるのではないかと考え、昨年度の研究において、重症心身障害児（者）８名とAADC欠損症1例について唾液中メラトニン濃度測定を行った。その結果は、重症児（者）のほとんどはメラトニン分泌は障害されておらず、むしろ過剰に分泌されており、AADC欠損症においては予想どおりメラトニン分泌が障害されていること、を示唆するものであった。

本年度は、さらに正常対照群を加え、重症心身障害児（者）とAADC欠損症についても症例を加えて検討した。

B．研究方法

対象は、東京都立東部療育センターに長期入所中の重症児（者）13名（以下重症児（者）群)、AADC欠損症の3名（７－14歳)、正常対照群5名（7－49歳）である。重症児（者）群は全例が寝たきりで寝返り不可の運動障害と言語理解や発語のない最重度精神遅滞の状態の合併がある。

唾液検体は、日中の11時から13時と夜間23時から1時に、直接吸引をして採取した。採取後は速やかに冷凍した。当センター到着後は－80℃の冷凍庫に移して測定まで保存した。唾液中メラトニンはDirect MELATONI ELISA キット(BUHLMAN社）を用いて測定した。その後、それぞれの群の夜間メラトニン濃度を比較検討した。また昼のメラトニン分泌抑制能力を示す値として、昼間メラトニン濃度と夜間メラトニン濃度の比を計算し比較した。検定はスチューデントのｔ検定を用いた。各群の統計値は、平均値±標準偏差であらわした。

本研究は、東京都立東部療育センターの倫理委員会にて承認を受けた。対象者の家族からは文書による承諾を得た。

なおAADC欠損症の唾液採取に関しては、山形大学　加藤光広先生にご協力いただいた。

C．研究結果

唾液中メラトニン濃度測定の結果を以下の通りだった。(図 1)

正常対照群：

夜 17.3±3.2 pg/ml, 昼 3.0±0.3 pg/ml

重症児（者）群

夜 31.4±16.1 pg/ml, 昼 17.6±13.2 pg/ml

AADC 欠損症

夜 4.8±3.5 pg/ml, 昼 4.7±3.9 pg/ml

AADC 欠損症の夜間の唾液中メラトニン濃度は、正常対照群や重症児（者）群と比べて有意に低下していた（P=0.002, 0.015)。

昼間メラトニン濃度と夜間メラトニン濃度の比は、正常対照群で 0.18±0.07 であり、昼間のメラトニン分泌は夜間の 2 割程度に抑制されていた。正常対照群の 2SD 以上を高値と考えると、重症児(者)群では 13 名中 9 名が高値をしめし, AADC 欠損症では 3 例ともが高値だった（0.37, 0.48, 3.44)。(図２)

D．考察

AADC 欠損症では、メラトニンの分泌が障害されていることが確かめられた。夜間唾液中メラトニン濃度は、重症児（者）群や正常対称群と比べて有意に低値であったため、重症児（者）の状態の症例に対してでも、AADC 欠損症のスクリーニング検査として有用であると考えた。

昼間と夜間の唾液中メラトニン濃度の比が、AADC 欠損症では高値の傾向があった。光情報を松果体に伝え、メラトニン合成を抑制する神経経路は交感神経を経由しているため、ノルエピネフリンの合成も障害される AADC 欠損症では、メラトニン合成抑制が障害されている可能性があると考えた。

E．結論

重症心身障害児（者）の中から AADC 欠損症の可能性のある症例をスクリーニングする方法として、夜間唾液中メラトニン濃度測定検査は有効である。

F．健康危険情報

なし

G．研究発表

1．論文発表

なし

2．学会発表

・重症心身障害児(者)の唾液中メラトニン測定。第 37 回日本重症心身障害学会学術集会　H23 年 9 月 29 日　徳島。(日本重症心身障害学会誌　36 巻、325 ページ、2011)

・ Salivary melatonin concentration in AADC deficiency. International AADC Conference, Selsdon Park Hotel, London , October 6th and 7th, 2011 (AADC 欠損症家族会の主催による国際会議)

H．知的財産権の出願・登録状況

1．特許取得　なし

2．実用新案登録　なし

3．その他　なし

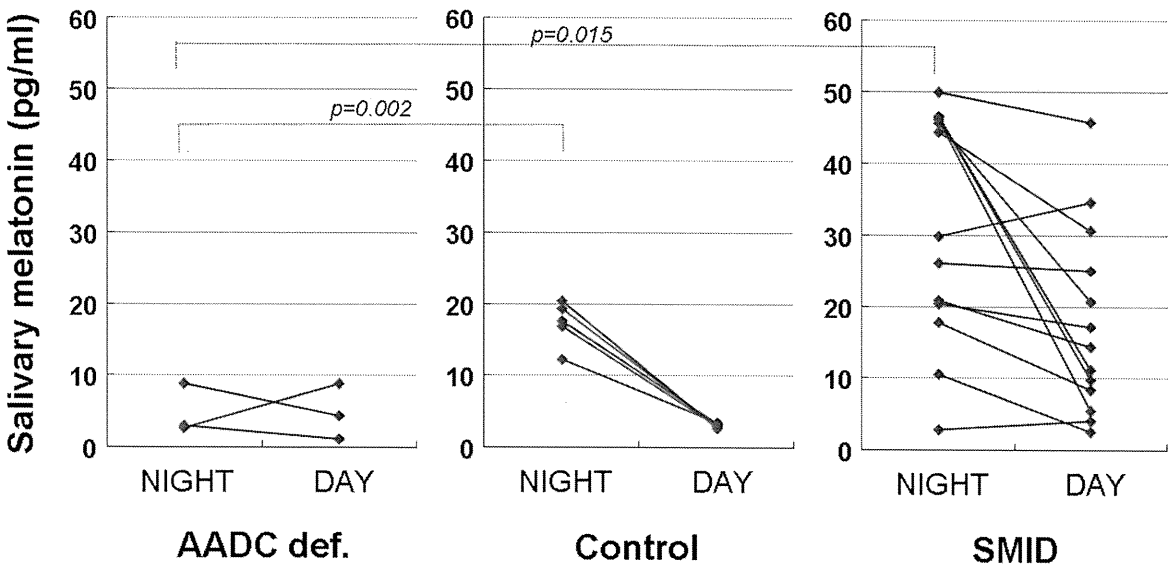

図１　唾液中メラトニン濃度の結果。夜間唾液中メラトニン濃度は AADC 欠損症群において、正常対照群や重症児(者)群よりも有意に低下している。 AADC def.=AADC 欠損症群、Control=正常対照群、SMID: 重症心身障害児（者）群

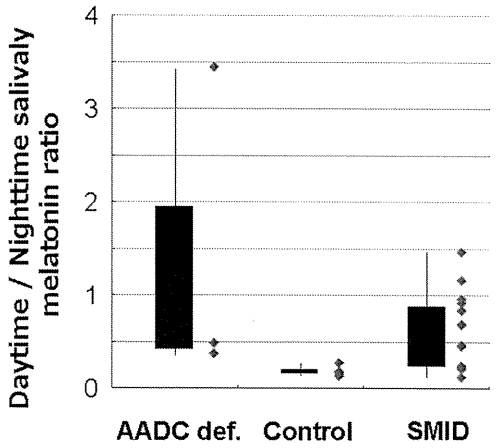

図２　日中唾液中メラトニンと夜間唾液中メラトニン濃度の比。AADC 欠損症群の３例ともが正常対照よりも高値であり、メラトニン分泌抑制機能の障害が疑われた。重症児（者）群の多くも高値を示した。

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

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研究成果の刊行物・別刷

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Hyperkinetic Movement Disorders
W.J. Weiner and E. Tolosa, Editors

Chapter 39

Dopa-responsive dystonia

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INTRODUCTION

In 1976 Segawa et al. reported a dystonia with marked response to levodopa as hereditary progressive dystonia (HPD) with marked diurnal fluctuation, which is now termed autosomal-dominant guanosine triphosphate cyclohydrolase 1 (AD GCH-1) deficiency (Segawa disease). Nygaard et al. (1988) proposed the term dopa-responsive dystonia (DRD) for this disorder. The criteria of Calne (1994) for DRD are the same as those for HPD, and DRD is now used to refer to all dystonias responding to levodopa. In this chapter the clinical characteristics and pathophysiologies of DRD are discussed by reviewing AD GCH-1 deficiency, recessive deficiencies of enzymes of pteridine metabolism, recessive tyrosine hydroxylase (TH) deficiency, DYT14, and juvenile parkinsonism (JP) caused by *PARKIN* gene (PARK-2).

AUTOSOMAL-DOMINANT GTP CYCLOHYDROLASE 1 DEFICIENCY (SEGAWA DISEASE): DOMINANT DYT5

AD GCH-1 is caused by mutation of the *GCH-1* gene, located on chromosome 14q22.1-q22.2. This disease was first reported as hereditary progressive basal ganglia disease with marked diurnal fluctuation in two girls (cousins) (Segawa et al., 1971). Segawa et al. (1976) also reported a 51-year-old woman with a movement disorder for 43 years (onset at 8 years of age) without treatment and confirmed this disease as dystonia. This is an autosomal-dominantly inherited generalized postural dystonia. The hallmarks of this disease include childhood onset, diurnal fluctuation of symptoms, and marked and sustained response to levodopa (Segawa et al., 1971, 1976; Segawa, 1981). Prior to the molecular identification of HPD, Deonna (1986) termed this disorder Segawa's syndrome because of the presence of a recessively inherited type. The latter, however, was revealed later to be recessive TH deficiency, which is now classified as recessive DYT5. After the term "DRD" was introduced by Nygaard et al. (1988) and by Calne (1994), the terms "DRD" or "HPD/DRD" were adopted instead of HPD in most English-language journals.

AD GCH-1 deficiency, or Segawa disease, is classified as dominant DYT5 since the discovery of the causative gene (Ichinose et al., 1994). The recessive DYT5 is due to TH deficiency. Although phenotypical variations had been shown in HPD (Nomura and Segawa, 1993), the discovery of the causative gene further clarified these variations (Bandmann et al., 1996, 1998). Some reports continue to use the term "Segawa syndrome" because of its broad phenotypical implications. In this chapter the clinical characteristics of AD GCH-1 deficiency are reviewed, including the pathophysiology, neuroimaging, neurophysiological, neuropathological, neurohistochemical, molecular biological studies, animal models, and pathophysiology.

Clinical signs and symptoms

The clinical features of AD GCH-1 deficiency are derived from the long-term study of 41 gene-positive patients from 20 families personally examined (Segawa, 1981, 2000a; Segawa et al., 2003).

In these 41 patients, 38 had onset in childhood (average age at onset 6.4 ± 2.7 years). The other three had adult onset at 28, 35, and 58 years. Family history suggested 8 additional cases in the parental generation of the probands, 2 with onset in childhood (2.6 and 5 years), and the rest in adulthood. Probable patients among grandparents exist with onset from 50 to 65 years. Anticipation occurs in some DRD families.

In childhood onset (38 cases) the initial symptom was equinovarus of one foot in 23 (60.5%) and gait

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disturbance in 12 (31.5%). Two started with hand tremor and 1 with dystonia of one arm. The initial symptoms of adult-onset patients were leg tremor at age 28 years, writer's cramp at 35 years, and hand tremor and rigidity at age 58. Tremor was observed in 16 (42%) of childhood-onset patients, but appeared after 10 years of age. If patients received levodopa before age 10 they did not show tremor throughout the course of illness. Two childhood-onset patients had retrocollis, and one developed oculogyric crises.

Diurnal fluctuation of symptoms was observed in all with childhood onset. The neurological symptoms became worse towards evening and recovered markedly in the morning after sleep. However, fluctuations were not apparent in adult-onset patients.

Some AD GCH-1 deficiency families exhibit postural dystonia and some dystonia with movements.

In my own 41 patients, the female-to-male ratio was 33:8, so it seems that AD GCH-1 deficiency is also characterized by gender preference. The gender difference reversed in adult-onset patients.

Neurological examination

Childhood-onset patients have rigid hypertonus, which fluctuates.

Postural tremor (8–10 Hz) and not a parkinsonian resting tremor is present. There is no cogwheel rigidity. Diadochokinesis is slow and clumsy, and pronation/supination movements of one arm induce rigidity in the contralateral arm. Tendon reflexes are exaggerated and ankle clonus may be observed. Plantar response is not extensor, although some show tonic dorsiflexion of the big toe (striatal toe sign). Gait is rigid akinetic, secondary to the rigid hypertonus caused by co-contraction of agonistic and antagonistic muscles in the lower extremities. Propulsion is observed in advanced disease; however, locomotion is preserved and freezing phenomenon or *marche à petit pas* is not seen. These clinical signs, particularly rigidity and tremor, are asymmetric.

Scoliosis is observed in all cases and it is convex to the side of predominantly involved extremities. Postural instability is observed, and is marked on the convex side of the spine. Camptocormia was not observed in my patients.

Cerebral and cerebellar dysfunction are not observed. Sensory disturbances are not detected.

Clinical course

The clinical symptoms of AD GCH-1 deficiency vary with age of onset and also show age-dependent variation. Typically, symptoms begin around 6 years of age with equinovarus involving one lower extremity. Around age 10, postural tremor may appear in one upper extremity. Postural dystonia spreads to all limbs by 10–15 years, with worsening of dystonic rigidity. With progression of dystonia, bradykinesia is observed in all and some have retropulsion, hypomimia, and dysarthria, but no patients have freezing of gait or failure of locomotion. Progression tends to be slower with age, and reaches a plateau by the fourth decade. Postural tremor continues to evolve and, by the fourth decade, it appears in all extremities and the neck. The diurnal fluctuation, particularly that associated with dystonia, declines with age. The fluctuations may not be apparent by age 20, and almost disappear by the fourth decade.

The clinical course also depends on age of onset. Patients developing symptoms in late childhood or early teens begin with dystonia of the upper limbs with or without postural tremor. Some have action dystonia of an upper extremity and/or retrocollis with or without oculogyric crises. During the course of the illness, patients with action dystonia may develop torticollis or writer's cramp in adulthood. Those with onset in adulthood start with hand tremor, torticollis, or writer's cramp, without postural limb dystonia and without diurnal fluctuation. Although there is mild rigidity, there is no progression. In older adults, generalized rigidity with postural tremor but without postural dystonia and diurnal fluctuations is the rule.

Exaggeration of tendon reflexes is a characteristic feature of patients with onset in childhood.

Asymmetry of symptoms is commonly observed throughout the illness without any relation to age of onset.

Clinical variation

Since the discovery of the causative gene, phenotypical variation of AD GCH-1 deficiency has greatly expanded (Bandmann et al., 1996, 1998). Phenotypic variation is further expanded by studies of early-onset cases (Lopez-Laso et al., 2007; Nagata et al., 2007; Cheyette et al., 2008), families with psychiatric disorders (Van Hove et al., 2006), compound heterozygotes (Furukawa et al., 1998a), and a case with dopa-responsive myoclonus-dystonia syndrome (Leuzzi et al., 2002). Considerable attention has been paid to whether postural or action dystonia is present, and to patients with postural hypotonia and psychological disorders.

Action dystonia is characterized by dystonic movements and focal or segmental dystonia in addition to postural dystonia (fixed). Before the *GCH-1* gene was discovered, action dystonia was thought to respond differently to levodopa than HPD, because of neurophysiological findings suggesting dopamine (DA) receptor supersensitivity (Nomura and Segawa, 1993).

Patients with action dystonia also showed paroxysmal dystonia, dystonic cramps, oculogyric crises, and focal or segmental dystonia. Adult onset is observed only in families with action dystonia and they may have writer's cramp or generalized rigidity with tremor but they do not have generalized postural dystonia.

Early-onset patients have hypotonia, particularly postural or truncal hypotonia, failure in development of locomotion, delay in motor and mental development in infancy, and autistic behavior in infancy and early childhood. These symptoms might be related to deficiency of 5-hydroxytryptamine (5HT) (Segawa, 2001; Segawa and Nomura, 2006).

The heterogeneity of symptoms seen in this disorder is related to age of onset and different pathophysiologic mechanisms at the DA neuron and involvement of 5HT neurons (Segawa et al., 2002).

However, not all phenotypic variability can be explained by these mechanisms, including levodopa-responsive myoclonus-dystonia (Leuzzi et al., 2002), and a patient with cerebellar signs (Chaila et al., 2006). In addition, bilateral resting tremor in the legs with mild leadpipe rigidity, and tremor associated with birth control pills was reported. This patient had two known polymorphisms in the *PARKIN* gene in addition to a mutation of *GCH-1* gene (Postuma et al., 2003).

Treatment and prognosis

In most cases, 20 mg/kg/day of levodopa without decarboxylase inhibitor (plain levodopa) completely alleviates the symptoms (Segawa et al., 1990, 2003). If decarboxylase inhibitors are used, the dose is 4–5 mg/kg/day. Some patients who started treatment with plain levodopa before 10 years of age develop decreased responsiveness around 13 years of age (Segawa et al., 1990). This is thought to be due to activation of dopa decarboxylase within the intestine around this age (Segawa et al., 1990). However, there are patients in certain families who continue to respond to plain levodopa throughout the course of illness. Adult-onset subjects do not necessarily respond to plain levodopa and treatment with a decarboxylase inhibitor is recommended. In a few cases, choreic movements developed after a rapid increase of levodopa dosage (Segawa et al., 2003). In compound heterozygotes aggravation of dystonia with the initial levodopa dosage is prominent (Furukawa et al., 1998a). In these patients, slow titration of levodopa to optimal doses results in favorable and sustained effects (Segawa et al., 1986, 1990; Furukawa et al., 1998a). In three of my own young-onset patients, levodopa was administered at the age of 38 years in two, and 51 years in the other. Levodopa resulted in marked and sustained benefits without side-effects for 8 and 37 years, respectively. Levodopa is effective for dystonia in almost all cases, independent of onset age and duration of disease (Segawa et al., 1990). Levodopa dose can often be reduced after age 30.

However, in patients with action dystonia the levodopa effect may not be complete, and in some cases retrocollis and oculogyric crises may be aggravated (Nomura and Segawa, 1993). Furthermore, patients with action dystonia developed infrequent levodopa-induced dyskinesia. One patient presented with JP (onset at 6 years) and later proved to have an action-type AD GCH-1 deficiency which responded markedly to levodopa but showed marked dopa-induced dyskinesia (Narabayashi et al., 1986). One patient, considered to have an action dystonia because of writer's cramp, developed levodopa-induced dyskinesia (Hjermind et al., 2006).

Anticholinergic drugs may have a marked and prolonged effect, but do not afford complete relief and may not improve tremor (Nomura et al., 1984). However, anticholinergic drugs with levodopa were helpful in patients with oculogyric crises (Segawa et al., 1990). Amantadine was beneficial for levodopa-related chorea (Furukawa et al., 2004). Bromocriptine was effective but did not give complete relief (Nomura et al., 1987). Tetrahydrobiopterin (BH_4) treatment was attempted on HPD/DRD patients (LeWitt et al., 1983a, b, 1986; Ishida et al., 1988; Ibi et al., 1991), in combination with levodopa. No favorable effects were obtained with BH_4 monotherapy (Ibi et al., 1991). In a compound heterozygote, co-administration of BH_4 with levodopa was necessary for complete recovery (Furukawa et al., 1998a).

Prior to the introduction of levodopa as a treatment, unilateral stereotactic pallidotomy and nucleus ventralis lateralis (VL) thalamotomy were performed in one patient with the onset of dystonia at age 6 years. She belonged to a family with postural dystonia and had the same mutation in *GCH-1* gene as other family members (Segawa et al., 1998). The pallidotomy performed at age 30 improved postural dystonia. Ipsilateral VL thalamotomy 7 years later in the same patient was effective for postural tremor. However, the effect of the pallidotomy on postural dystonia was incomplete and the VL thalamotomy showed no further beneficial effects on postural dystonia that remained. Levodopa started at age 41 showed complete and sustained improvement of the dystonia on the nonoperated side, but incomplete effects on the operated side. In a patient with action dystonia who presented as JP (Narabayashi et al., 1986), unilateral VL thalamotomy performed at age 24 improved the dystonic rigidity. Levodopa started at age 30 showed dramatic

effects but induced severe dyskinesia which occurred 1 year later on the nonoperated side. VL thalamotomy performed at age 38 was effective and led to prolonged and sufficient effects of levodopa.

Investigations

Biochemical studies

Cerebrospinal fluid (CSF) examination of catecholamine metabolites demonstrated low levels of homovanillic acid (HVA) throughout the day, with lower levels in the afternoon (Ouvrier, 1978; Kumamoto et al., 1984; Shimoyamada et al., 1986; Maekawa et al., 1988). Both biopterin and neopterin levels in CSF of patients with HPD (mutation of *GCH-1* gene) were markedly reduced below 20% of the normal range (LeWitt et al., 1986; Fink et al., 1988; Fujita and Shintaku, 1990; Furukawa et al., 1993). A 30–50% reduction of these substances was observed in CSF of asymptomatic carriers (Takahashi et al., 1995).

Activities of GCH-1 in mononuclear blood cells of patients were less than 20% of normal, while activity in asymptomatic carriers reached 30–40% of normal (Ichinose et al., 1994). Phenylalanine loading in both children and adults suggested decreased liver phenylalanine hydroxylase activity due to defective BH_4 (Hyland et al., 1997).

Neuroimaging

Magnetic resonance imaging (MRI) and computed tomography scans of the brain showed no abnormalities, while positron emission tomography (PET) demonstrated normal or low normal [^{18}F] dopa uptake (Sawle et al., 1991; Snow et al., 1993; Turjanski et al., 1995). Patients with onset at older ages (Okada et al., 1993) and asymptomatic carriers (Takahashi et al., 1994) showed normal levels. [^{11}C] Raclopride PET showed normal activity in symptomatic subjects (Leenders et al., 1995), and [^{11}C] N-spiperone PET revealed mild increase in receptor binding (Kunig et al., 1988; Kishore et al., 1998). However, no increase in receptor binding was demonstrated in follow-up PET analysis after 7 months of levodopa therapy (Kishore et al., 1998).

Three patients with action dystonia, two women with a 30-year clinical course after onset at 8 years old, and a man aged 59 with onset at 58 years had normal [^{18}F] dopa uptake and [^{11}C] N-spiperone PET.

These results suggest a functional deficit of DA metabolism, specifically a decrease in the hydroxylation of tyrosine with preservation of aromatic acid decarboxylase activity (Snow et al., 1993). Increased D_2 receptor binding is not considered a factor in determining the clinical state of AD GCH-1 deficiency (Kishore et al., 1998). Furthermore, [^{123}I] ß-CIT single-photon emission computed tomography (SPECT) scanning is normal, suggesting that the DA transporter molecule does not seem to play a role in this disease (Jeon et al., 1998).

However, Kikuchi et al. (2003) demonstrated decreased [^{18}F] dopa uptake in an older-onset case with a heterozygous recessive mutation of a gene for recessive type. Hjermind et al. (2006) showed decreased presynaptic D_1 receptor uptake by demonstrating decreased [^{123}I]-*N*-w-fluoropropyl-2β-carbomethoxy-3β-(4-idiophenyl) nortropane (^{123}I-FP-CIT) binding rates with SPECT and speculated there was progressive loss of nigral cells in "early-onset Parkinson disease" with *GCH-1* gene mutation.

Neurophysiological studies

Polysomnography in patients with HPD who were later shown to have a heterozygous mutation of the *GCH-1* gene revealed abnormalities of the phasic components of sleep. These changes included a decrease in the number of gross movements and twitch movements (TMs). Sleep structure, percentage sleep stages, and other parameters modulated by brainstem aminergic neurons were normal (Segawa et al., 1976, 1987, 1988; Segawa and Nomura, 1993). The number of TMs during rapid-eye-movement (REM) sleep (sREM) reflects neuronal activity of the nigrostriatal (NS) DA neuron (Segawa et al., 1987, 1988). This derives from results of selective sleep stage deprivation studies. Selective sREM deprivation dissolved morning recovery, while selective stage IV deprivation accentuated sleep effects with increased TMs in sREM (Segawa et al., 1976). In normal younger children the numbers of TMs in sREM are high and show an age-dependent decrement. Moreover, the TMs in sREM show incremental variation with sleep cycles. In AD GCH-1 deficiency these age and nocturnal variations of TMs were preserved, but with levels approximately 20% of normal (Segawa et al., 1988; Segawa and Nomura, 1993).

Abnormalities in the pattern of gross movements, that is, the rate of occurrence against sleep stages, differed between postural dystonia and action dystonia. In action dystonia, the pattern observed was similar to patients with D_2 receptor supersensitivity (Segawa et al., 1988). After levodopa treatment, abnormalities improved in the postural group, but not in the action group, even though they improved clinically (Segawa et al., 1988).

These findings suggest that the abnormalities of motor components of sleep observed in AD GCH-1 deficiency are related to a nonprogressive DA deficiency of the NS DA neuron.

Supracranial magnetic stimulation was normal, showing preservation of the corticospinal tract (Muller et al., 1989).

Paired pulse transcranial magnetic stimulation was reported (Huang et al., 2006; Hanajima et al., 2007). One study showed residual abnormalities in motor inhibition in levodopa-treated DRD patients even though they were clinically asymptomatic (Huang et al., 2006). The other study examined eight gene-positive and one biochemically confirmed GCH-1 deficiencies and revealed that dysfunction of GABAa-inhibitory interneurons of the primary motor cortex does not contribute to the generation of dystonia of AD GCH-1 deficiency (Hanajima et al., 2007).

Studies from our group on gating process with sensory evoked potentials revealed normal gating in patients with postural dystonia, while it was abnormal in patients with action dystonia.

Evaluation of saccades revealed abnormalities in both visually guided saccades and memory-guided saccades, and implicates both the direct and the indirect pathways (Hikosaka et al., 1989, 1993). One study revealed that in adult-onset patients belonging to families with action dystonia, abnormalities were observed only in memory-guided saccades.

These results implicate AD GCH-1 deficiency in the thalamocortical pathways. GABAa inhibitory interneurons are not affected in postural dystonia but are affected in action dystonia. The indirect pathways are involved in action dystonia and symptoms observed in adult-onset cases.

Brain pathology and histochemistry

Neuropathological and neurohistochemical study was available on a 19-year-old woman with DRD who died in a traffic accident (Rajput et al., 1994; Hornykiewicz, 1995), and was later proven to be AD GCH-1-deficient by DNA analysis (Furukawa et al., 1996). Gross inspection failed to demonstrate any changes in the substantia nigra (SN) beyond decrease in the melanin, particularly in the ventral tier of the pars compacta of the SN (SNc). Histochemically, DA content was subnormal in the SNc. The magnitude of the striatal DA loss was not as severe as in Parkinson's disease (PD) but clearly below the lower limit of the control range. The reduction was greater in the putamen than in the caudate nucleus and marked in the rostral caudate and the caudal putamen, similar to PD. However, in contrast to PD, this case showed a greater DA loss in the ventral subdivision of the rostral caudate than its dorsal counterpart, though in the putamen, the dorsoventral DA gradiant was similar to PD. Furthermore, the activity and protein content of TH were decreased in the striatum, while they were within the normal range in the SNc (Hornykiewicz, 1995).

Neuropathology in a patient with action dystonia presented as JP (Narabayashi et al., 1986) showed hypomelaninized and round-shaped immature DA neurons in the SNc. A few Lewy body-like bodies were observed in the SNc and the locus ceruleus. Neurohistochemisty revealed decreased DA in the striatum with predominance in the putamen but no significant decrement in the SNc.

Furukawa et al. (1999) reported similar neuropathological findings in two brains. Although the DA content in the striatum was not reported, these investigators did show marked reduction of total biopterin (84%) and neopterin (62%) in the putamen, despite normal concentration of aromatic acid decarboxylase, DA transporter, and vesicular monoamine transporter. Additional postmortem study of an asymptomatic carrier by these investigators revealed modest reduction of TH protein (52%) and DA (44%), despite marked reduction of striatal biopterin (82%), and postulated that the levels of TH protein were a key for symptomatic or asymptomatic individuals (Furukawa et al., 2002).

Molecular biological studies

Nygaard et al. (1993) mapped the DRD locus to a 22cM region, between D14S47 (14q11.2-q22) and D14S63 (14q11-q24.3) on chromosome 14q. Ichinose et al. (1994) found the gene *GCH-1* by examining seven patients with HPD (Segawa disease) and demonstrated the mutation *GCH-1* located on 14q22.1-q22.2. More than 100 independent mutations have been identified in the coding region of *GCH-1* (Ichinose et al., 1994; Bandmann et al., 1996; Nishiyama et al., 2000).

Extensive genetic evaluation, combined with CSF study of biopterin and neopterin levels and mononucleocyte GCH-1 levels in asymptomatic carriers, confirmed that HPD/DRD is an autosomal-dominantly inherited GCH-1 deficiency with low penetrance (Ichinose et al., 1994; Nygaard et al., 1994; Takahashi et al., 1994).

Molecular analysis remains unable to determine mutations in the coding region of the gene in approximately 40% of subjects with GCH-1 deficiency (Furukawa and Kish, 1999). In some of these subjects abnormalities in intron genomic deletion (Ichinose et al., 1995; Nishiyama et al., 2000), a large gene deletion (Furukawa et al., 2000; Wider et al., 2008), an intragenic duplication or inversion of *GCH-1* or mutation in as yet undefined regulatory gene-modifying enzyme function (Furukawa and Kish, 1999) may be present.

Pathophysiology

Although the pathogenetic mechanisms for dominant inheritance are unknown, classic dominant negative (Hirano et al., 1998; Hwu et al., 2000) and destabilizing effects (Suzuki et al., 1999) have been considered to explain presenting symptoms with heterozygous mutations. The wide variations in clinical expression may depend on a number of factors, including the locus of the genetic mutation and the ratio of mutant versus normal gene in the area of active neurological substrate. The rate of mutant GCH-1 messenger ribonucleic acid (mRNA) production against normal RNA was 28% in a patient and 8.3% in an asymptomatic carrier (Hirano et al., 1995, 1996). However, the ratio varies depending on the locus of the mutation (Hirano et al., 1996; Suzuki et al., 1999; Ueno and Hirano, 2000). Furthermore, the ratio differed among affected individuals in some families, depending on the locus of the mutation (Hirano et al., 1996; Ueno and Hirano, 2000). This suggests that the degree and the pattern of inactivation of normal enzyme by mutant gene depend on the locus of mutation (Hirano et al., 1996; Ueno and Hirano, 2000) and may cause inter- and intrafamilial variation of the phenotype and penetrance. Loci of mutation for postural and action dystonia observed in our patients are shown in Figure 39.1.

GCH-1 is the rate-limiting enzyme for the synthesis of BH_4, the co-enzyme for synthesis of TH, and also tryptophan hydroxylase (TPH). In AD GCH-1 deficiency TH is preferentially affected when compared to TPH. This could be explained by the difference in distribution of GCH-1 mRNA in DA and 5HT neurons (Shimoji et al., 1999) or destabilization of the TH molecule or impairment of axonal transport (Furukawa, 2003). There is also a difference of K_m value for TH and TPH (Davis et al., 1992). In a heterozygous mutant gene BH_4 partially decreases in AD GCH-1 deficiency. TH with higher affinity to BH_4 is affected selectively. However, in molecular conditions with marked decrease of BH_4, TPH is affected as well as TH.

The loss of striatal TH protein with preservation in the SNc might be explained by BH_4 controlling protein stability rather than expression (Furukawa et al., 1999). Leff et al. (1998) presented gene transfer data and suggested a role for stabilization of TH protein by co-expression of GCH-1 *in vivo*. Sumi-Ichinose et al. (2001) showed loss of TH protein but not of TH mRNA in the brains of BH_4-deficient mice.

Complete and sustained response to levodopa without relation to duration of clinical course suggests the absence of morphological or degenerative changes and suggests the lesion in AD GCH-1 deficiency is a nonprogressive functional lesion restricted to the NS DA neurons (Segawa, 1981; Segawa et al., 1986). Onset in the first decade of life with an age-related clinical course which correlates with age variation of the activity of TH in the synaptic terminals of the caudate nucleus of the NS DA neurons was shown by McGeer and McGeer (1973).

Activity of the NS DA neurons also shows circadian oscillation in the terminals (McGeer and McGeer, 1973). AD GCH-1 deficiency seems to be caused by nonprogressive loss of TH in the terminals of the NS DA neuron and clinical symptoms develop following

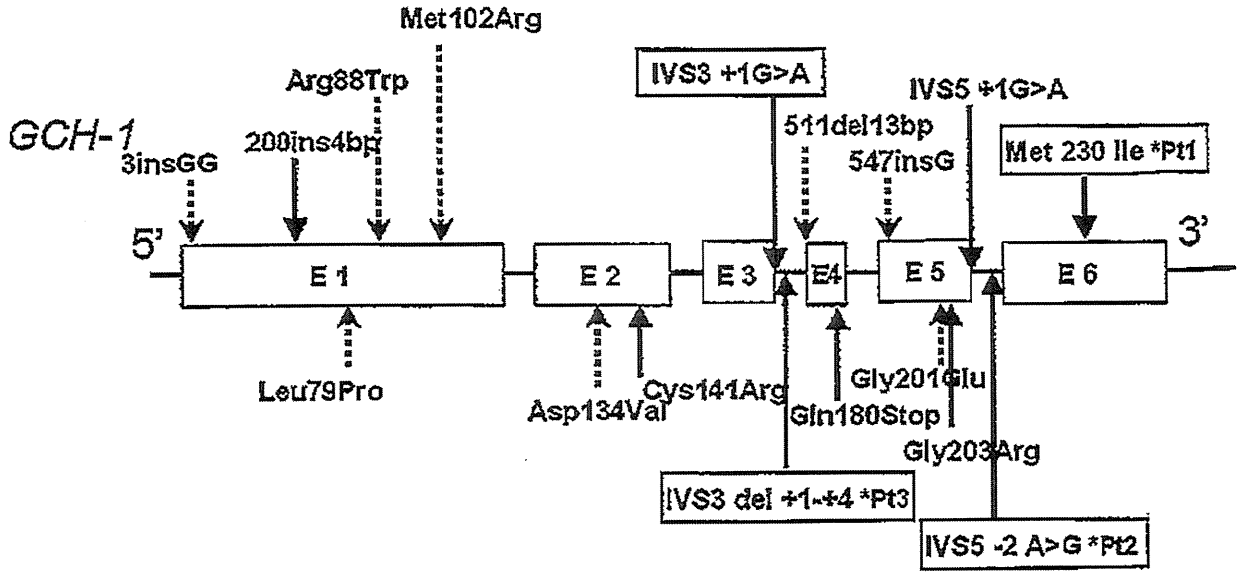

Fig. 39.1. Loci of the mutations of the guanosine triphosphate cyclohydrolase 1 (*GCH-1*) gene of my patients with autosomal-dominant GCH-1 deficiency. Solid arrows are postural dystonia. Dotted arrows are action dystonia. Mutations enclosed by a rectangle are those with adult-onset patients.

the age and circadian variation of TH activity of the terminals.

Compartmental substructure studies of the human striatum revealed that within the rostral caudate in particular, the ventral/medial portions, the striosomes/patches or D_1-direct pathways are more numerous, whereas in the dorsal/lateral portions, the matrix compartment is more homogeneous (Graybiel and Ragsdale, 1978; Gibb, 1996). Histochemical examination of a patient with AD GCH-1 deficiency by Hornykiewicz (1995) suggested that the DA deficiency is more prominent in the striosomes/patches compartment.

The most active stage of striosomal TH is in the fetal period and suggests high TH activity in the striosome has an important function for neuronal systems in the fetus or early infancy. Decreased TH in the striosome might relate to morphological disturbances of the SNc observed in AD GCH-1 deficiency (Narabayashi et al., 1986; Rajput et al., 1994). The decrease of ^{123}I-FP-CIT SPECT, observed in a patient reported by Hjermind et al. (2006), is not observed in PD. Suspected loss of nigral cells might be the result of failure in morphogenesis caused by hypofunction of the striosome-SNc pathway early in the developmental course. Dystonia is perhaps related to the altered physiology induced by these changes.

The striosome also has D_1 receptors, GABA, and substance P, that is, the striatal direct pathway which projects to the internal segment of the globus pallidus (Gibb, 1996).

Therefore, in childhood, a decrease of DA in the ventral area of the striatum causes dysfacilitation of the direct pathway, and inhibits the reticulospinal tract through disinhibiting its descending output. This may induce co-contraction of the agonistic and antagonistic muscles, which manifests clinically as postural dystonia.

Kreiss et al. (1996) showed that the NS DA neuron mediates the subthalamic nucleus (STN) via the D1 receptor located on the nucleus. The STN matures functionally in early infancy among the structures comprising the striatal indirect pathways (Kobayashi et al., 2005). Thus, for these symptoms the DA neuron innervating the STN with the D1 receptor is postulated to be involved (Segawa and Nomura, 1991; Segawa et al., 2002).

Particular findings of PSG in action type, aggravation of action dystonia by L-dopa, and effects of VL thalamotomy on tremor are explained by the STN lesion. Recent unpublished studies showing the effect of D3 agonist rather than L-dopa on parkinsonism observed in a family of action type, and dominantly inherited restless-leg syndrome in a family with heterozygous abnormalities of *GGCH*-1 gene support the involvement of the STN on action-type Segawa disease.

DYT1 dystonia can also have postural and action components, depending on the family (Nomura et al., 2000). Based on the effective foci of the stereotactic operation (Lenz et al., 1992; Shima et al., 1995), postural dystonia is considered to be caused by a lesion of the striatal direct pathway and the descending output of the basal ganglia, while the movement component or action dystonia is related to the striatal indirect pathway and the ascending output of the basal ganglia. DYT1 may have very similar pathophysiology in the basal ganglia as AD GCH-1 deficiency, with phenotypical variation depending on the family.

This postulates that the striatal pathways and the outputs of the basal ganglia have different developmental courses independent of the NS DA neurons, and the age-related emergence of symptoms in AD GCH-1 deficiency depends on the development of the striatal pathways.

The pathophysiology of AD GCH-1 dystonia is shown in Figure 39.2.

There remain inconsistencies in trying to explain all the phenotypic variations. For example, an adult-onset DRD patient with parkinsonism was reported to have a heterozygous mutation of *GCH-1*. This had only been reported in a heterozygous mutation of the gene for recessive GCH-1 deficiency with hyperphenylalanemia (Kikuchi et al., 2003). ^{18}F-fluorodopa uptake in this patient was significantly decreased in caudate and putamen. Borderline to below-normal IQ score observed in this patient suggests more widespread involvement of other neuronal structures. Cerebellar dysfunction (Chaila et al., 2006) is also difficult to explain from the pathophysiologies discussed.

Gender difference is also difficult to explain. Ichinose and colleagues (1994) examined the basic levels of GCH-1 in mononuclear blood cells and found higher levels in males. Furukawa and colleagues (1998b) showed a much higher penetrance in females (87%) than in males (38%), and similar results have been observed in my 47 subjects.

Thus, marked female predominance might depend on a genetically determined gender difference of the DA neuron (Reisert and Pilgrim, 1991).

Diagnosis

Diagnosis of AD GCH-1 deficiency is usually not difficult when characteristic clinical symptoms are present. The phenylalanine loading test is helpful, but may yield false-negative results. Gene analysis for the mutation of *GCH-1* gene is the most definitive test. However, 20–40% of these tests were reported to be negative and therefore biochemical studies are recommended. Determination of GCH-1 activity in peripheral

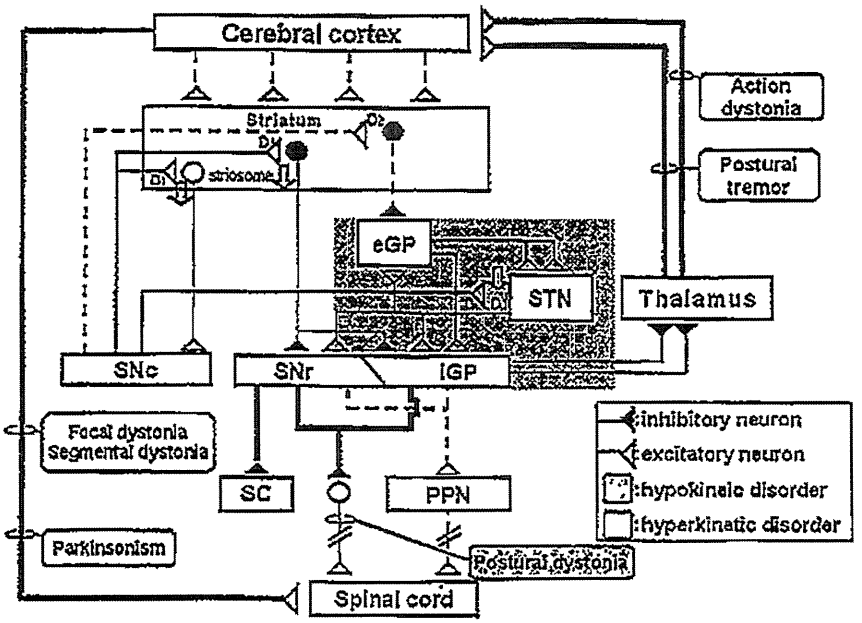

Fig. 39.2. Pathophysiology of autosomal-dominant guanosine triphosphate cyclohydrolase 1 (GCH-1) deficiency. eGP, external segment of globus pallidus; iGP, internal segment of globus pallidus; STN, subthalamic nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SC, superior colliculus; PPN, pedunculopontine tegmental nucleus. Symbols: Solid, single lines are pathways involved in pathophysiology. The width shows degree of activities. Broken lines are pathways not involved in pathophysiology. Closed triangles are inhibitory neurons. Open triangles are excitatory neurons. Shaded region with dots is the suggested area of the circuit for postural tremor. GABAergic neurons of the striosome-SNc show the state of fetus period to early infancy, i.e., excitatory. Symptoms in an open rectangle are those of excitatory disorders, and in a gray rectangle are those of inhibitory disorders.

mononucleated cells is the most reliable, but technically complicated. Because of this, determination of neopterin and biopterin levels in CSF is most reliable for diagnosis.

Differential diagnosis

All children with a gait disturbance and limb dystonia should be evaluated for AD GCH-1 deficiency. The differential diagnosis includes Wilson's disease, brain degeneration with iron accumulation, hereditary spastic paraplegia, and cerebral palsy. AD GCH-1 deficiency is often misdiagnosed as hereditary spastic paraplegia. Initial diagnosis in some patients included Duchenne muscular dystrophy, psychological reaction, or hysteria. The differentiation of AD GCH-1 deficiency from these disorders is usually not difficult with clinical examination.

Childhood-onset patients can often be differentiated from dopa-nonresponsive dystonia by the absence of axial torsion. Segawa suggests that predominant involvement of the sternocleidomastoid muscle (SCM) contralateral to limb involvement is indicative of AD GCH-1 deficiency, while ipsilateral SCM involvement is seen in dystonias with axial torsion (Segawa et al., 1998, 2002; Segawa, 2000a, b, 2002).

In adult-onset AD GCH-1 deficiency, torticollis ipsilateral to the side of the predominantly affected extremities is seen. Thus, torticollis and writer's cramp without generalized dystonia in adult-onset AD GCH-1 deficiency are difficult to differentiate from dopa-nonresponsive dystonia by clinical examination. Exaggeration of deep tendon reflexes, ankle clonus, and striatal toe may lead to the misdiagnosis of spastic paraplegia. Careful family history and the levodopa challenge test are recommended. Definite diagnosis is dependent on gene analysis and/or biochemical studies.

DRDs other than AD GCH-1 deficiency include recessive disorders of the enzymes of pteridine metabolism and recessive TH deficiency (recessive DYT5). All of the inherited disorders of pteridine metabolism develop levodopa-responsive dystonia caused by decrease of BH_4 or TH in infancy and early childhood as in AD GCH-1 deficiency (Nomura et al., 1998). Although these patients have marked postural hypotonia, failure of development, and disturbance of psychomental function secondary to 5HT or norepinephrine deficiency, these phenotypes can lead to misdiagnosis of AD GCH-1 deficiency.

All JP patients have features of DRD. All have parkinsonism, including rigidity and/or resting tremor. However, if symptoms begin in childhood or early

teens, dystonia is seen. Dystonia of JP responds markedly to levodopa, but dyskinesia develops soon after levodopa is started. JPs, particularly those caused by the *PARKIN* gene (PARK 2), are particularly important to differentiate from AD GCH-1 deficiency.

Some patients with AD GCH-1 deficiency may develop parkinsonian symptoms in late adulthood. In these patients tremor and gait disturbance are the primary signs, and dystonia is absent or not prominent and diurnal fluctuation is not observed (Nomura and Segawa, 1993; Segawa, 2000b). These patients are often misdiagnosed as JP or PD (Nomura and Segawa, 1993; Segawa et al., 1986, 2002). However, the tremor in these cases is mainly postural, their clinical features are milder, and there is no progression after levodopa is started. There is no cogwheel rigidity and [^{18}F] dopa PET and [^{11}C] spiperone PET scan in these late-onset adult cases reveal normal uptake, as seen in childhood-onset AD GCH-1 deficiency (Segawa et al., 2003).

RECESSIVE DEFICIENCY OF THE ENZYMES OF PTERIDINE METABOLISM

Metabolic maps of pteridine metabolism and the processes for synthesis of DA are shown in Figure 39.3. The enzymes, deficiency of which causes DRD, are shown in the wide rectangles in this figure. The basic pathophysiologies are considered the same as those of AD GCH-1 deficiency.

Recessive GTP cyclohydrolase 1 deficiency

Recessive GCH-1 deficiency is rare among BH_4 deficiencies (Blau et al., 1996). This disease was initially reported in infants with severe motor and mental retardation, hypotonia of the trunk and the extremities, convulsions and frequent episodes of hyperthermia without infection (Niederwieser et al., 1984). Diagnosis is confirmed by marked decrease of neopterin, biopterin, pterin, isoxanthine, DA, and 5HT in the urine, and decreased HVA, 5-hydroxyindole acetic acid (5-HIAA), neopterin, and biopterin in CSF (Niederwieser et al., 1984). Treatment with L-erythro BH_4 is effective, but the D-erythro tetrahydropterin form does not produce clinical benefit (Niederwieser et al., 1984).

Recessive pyruvoyl-tetrahydropterin synthase deficiency

6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is one cause of hyperphenylalaninemia (HPA) (Dudesek et al., 2001). According to the international database of BH_4 deficiencies, PTPS deficiency is the most common

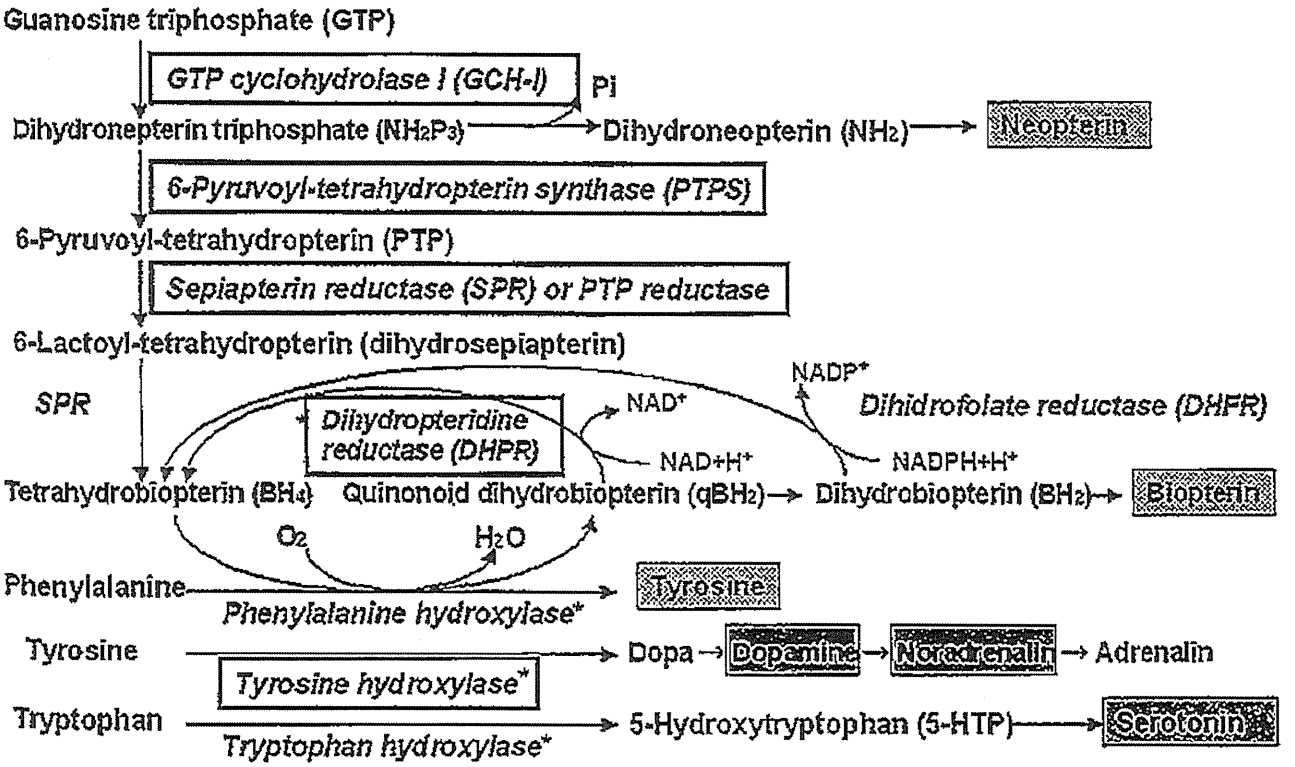

Fig. 39.3. Tetrahydrobiopterin (BH_4) and aromatic amino acid hydroxylase metabolism system. Enzymes involved in dopa-responsive dystonia are shown in open wide rectangles. (Reproduced from Shintaku H (2009) [Metabolic disorders of phenylalanine.] Jpn J Pediatr Med 41 (Suppl.), 334–340.)

form, representing approximately 60% of cases. This disorder is frequently observed in Taiwanese Chinese (Liu et al., 2001), and the prevalence is higher in Taiwanese (1/132 000) (Chien et al., 2001) than in white individuals (1/1 000 000) (Curtius et al., 1991).

Symptoms appear in infancy with delay in motor and mental development, hypotonia, hypersalivation, dysphasia, abnormal involuntary movements, and seizures. Limb dystonia with increase in muscle tone becomes apparent later in childhood and choreic movements appear in late childhood or adolescence. Diurnally fluctuating dystonia is observed in the eyelids, oromandibular region, and trunk (Roze et al., 2006). In contrast to AD GCH-1 deficiency, this fluctuation is observed even in adults over 30 (Hanihara et al., 1997). Diagnosis is by urine high-performance liquid chromatography analysis to demonstrate increased neopterin and decreased biopterin.

Levodopa with BH_4 (Hanihara et al., 1997; Roze et al., 2006) and levodopa with BH_4 and 5-hydroxytryptophan (5HTP) (Demos et al., 2005) produced dramatic and sustained effects. However, Tanaka et al. (1989) observed on–off phenomenon in a 10-year-old Japanese girl after oral levodopa (2 mg/kg/day). The motor fluctuations improved after continuous intravenous infusion of levodopa at plasma concentrations in 120–150 mg/dL. BH_4 supplementation with restriction of high-protein foods reduced HPA, but does not improve motor symptoms, including choreoathetoid movements and failure of interlimb coordination (Roze et al., 2006).

Because 5HTP is associated with improvement of cognitive testing, early treatment in combination with BH_4, levodopa, and 5HTP is recommended (Chien et al., 2001).

A long-term follow-up study of 12 PTPS deficiency patients revealed the importance of early treatment (before 1 month of age) with BH_4, 5HTP, and levodopa for preserving normal IQ scores (Liu et al., 2008). Levodopa administered with decarboxylase inhibitor (10–15 mg/kg/day) resulted in no dyskinesias.

Molecular genetics

Forty-three mutant alleles associated with deficiency of DA and 5HT have been identified on chromosome 11q22.3-q23.3 (Oppliger et al.; 1997). A patient with the homozygous K219E allele had transient HPA (Thony and Blau, 1997). Liu et al. (2008) showed N52S/P87S phenotype is benign. Upon co-transfection of two PTPS alleles, the N47D allele had a dominant negative effect on both the wild-type PTPS and the D116G mutant (Scherer-Oppliger et al., 1999). This suggests the possibility of the existence of autosomal-dominant PTPS deficiency.

Pathophysiology

In PTPS deficiency the decrease in activity of TH caused by BH_4 deficiency is thought to be restricted to the terminal of the NS DA neuron, as in AD GCH-1 deficiency. This is suggestd by an animal model of a homozygous defect of the PTPS gene, resulting in PTPS deficiency in mice (Sato et al., 2008). The decrease of DA activity in PTPS deficiency may have no effects on development of mental activity, while mental retardation, hypotonia, and failure in locomotion may be related to 5HT deficiency. Early replacement of DA and 5HT before 1 month of age may lessen the poor outcome of this disorder.

Recessive sepiapterin reductase deficiency

Bonafe et al. (2001) reported two patients with progressive early-childhood psychomotor retardation and dystonia associated with severe reduction of 5-HIAA and HVA and high levels of biopterin and dihydrobiopterin in the CSF. These children exhibit normal urinary pterins without HPA. Analysis of skin fibroblasts clarified inactive sepiapterin reductase (SPR), which was confirmed by mutations in the *SPR* gene located on chromosome 2p14-p12.

Neville et al. (2005) presented an additional seven patients with two pairs of siblings from Malta. All presented with motor delay in infancy and cognitive impairment with delay in language. Oculogyric crises were observed in six between 2 months and 1 year of age. Two exhibited retrocollis and dystonia was observed in five others. In infancy, hypotonia was predominant in these patients. Even in the two with dystonia in infancy there was truncal hypotonia. Four had chorea, two before levodopa and two after. All had diurnal variation, with sleep providing a restoration of some motor skills and a temporary cessation of oculogyric crises.

In all, the motor symptoms showed dramatic response to levodopa with decarboxylase inhibitor. Bulbar function, ocular manifestations, and tremor improved completely. Although all became able to walk, the pattern was not normalized. Usually minor motor manifestations of chorea and dystonia persisted and in some levodopa tended to aggravate chorea. One with chorea before levodopa had writer's cramp after levodopa. Levodopa never restored normal development or educational progress.

A 14-year-old girl initially presented at the age of 2 years with hypotonia and mild cognitive delay. She became wheelchair-bound at the age of 6 and dystonia became apparent at 14 (Abeling et al., 2006). These symptoms improved dramatically with levodopa and 5HTP started at age 14.

MOLECULAR BIOLOGY AND PATHOPHYSIOLOGY

Molecular studies on the Maltese population found two mutations, the c.68G>A in quinoid dihydropteridine reductase (*QDPR*) (p.G23D), and the new *SPR* mutation, IVS2-2A>G, at the splice site consensus sequence in intron 2 of the *SPR* gene, as causative mutations in all the patients with *SPR* deficiency and dihydropteridine reductase (DHPR) deficiency (Farrugia et al., 2007). All patients were heterozygotes for the corresponding mutation and showed no clinical symptoms. Three polymorphisms, c.96C>T (p.A32A), c.345G>A (p.S115S), and c.396G>A (p.L132L), have also been identified in the *QDPR* gene, defining four wild-type frameworks, useful in molecular epidemiology studies. The c.68G>A mutation in *QDPR* was found only on framework I, suggesting a founder effect. In contrast no additional sequence diversity was found in the *SPR* gene, whether in wild-type or mutant alleles, which is also consistent with a founder effect.

Deficiency of *SPR* affects the alternative pathways of the cofactor BH_4 via carbonyl, aldose, and dihydrofolate reductase. As a consequence of the low dihydrofolate reductase activity in the brain, dihydrobiopterin intermediate accumulates in the brain and inhibits TH and TPH and uncouples nitric oxide synthase, leading to deficiency of DA and 5HT and possibly to neuronal cell death (Blau et al., 2001), while high dihydrofolate reductase in peripheral tissues HPA does not occur.

Very low levels of HVA and 5HIAA and high levels of biopterin and sepiapterin in the CSF are the diagnostic hallmarks (Abeling et al., 2006).

Although neuropathology or neurohistochemistry has not been clarified, similar pathology to AD GCH-1 deficiency might exist for enzyme deficiencies of pteridine metabolism. Marked hypotonia in infancy and failure of development of locomotion might be due to 5HT deficiency.

At a molecular level, a single gene defect causes *SPR* deficiency (IVS-II, 2G) and mutation of a second nucleotide in the second exon–intron junction is thought to impair transcription processing and diminish sepiapterin mRNA levels (Blau et al., 2001; Farrugia et al., 2007).

Recessive dihydropteridine reductase deficiency

Although the incidence of recessive DHPR deficiency among BH_4 deficiencies is not small (Blau et al., 1996), case reports of recessive DHPR deficiency are rare. A Japanese boy reviewed by Nomura et al. (1998) became symptomatic at 2 months of age with dystonic postures which worsened towards evening or with long wakening periods. His motor development was delayed and he was able to sit at 1 year. Mental retardation was observed. Muscle tone was hypotonic at 3 years. Anticholinergic drugs aggravated these symptoms but the dystonic movements improved transiently with levodopa. At age 14, epilepsy developed. Marked and sustained improvement was seen with the co-administration of BH_4 and levodopa. However, there have been negative reports of BH_4 on recessive DHPR deficiency patients with mutant DHPR molecules (Cotton et al., 1986). Biochemical examination of blood showed moderate HPA and marked increase of plasma biopterin with normal neopterin levels. CSF examination showed below-normal neopterin and normal levels of biopterin and marked decrease of HVA and 5HIAA levels.

Deficient acitivity of DHPR is due to mutations in the *QDPR* DHPR gene on 4p15.31 (Farrugia et al., 2007). It results in defective recycling of BH_4 and homozygotes have a rare form of atypical HPA and phenylketonuria.

The pathophysiology may be similar to other enzyme deficiencies of pteridine metabolism. The DA and 5HT system may be affected. Early administration of levodopa and BH_4 is essential for treatment.

RECESSIVE TYROSINE HYDROXYLASE DEFICIENCY

Recessive TH deficiency (Castaigne et al., 1971) was demarcated from DRD or JP by Rondot et al. (1983). Deonna (1986) described this disorder as a recessive type of HPD. Knappskog et al. (1995) demonstrated the mutation of the TH gene located on chromosome 15. After the discovery of the gene, Hoffmann et al. (2003) suggested that the first or leading symptom is not dystonia but a progressive encephalopathy affecting several cerebral and possibly cerebellar systems.

Clinical characteristics

According to Hoffmann et al. (2003), the onset of progressive encephalopathy is much earlier than levodopa-responsive dystonia. Subtle diurnal fluctuations are observed. There may be cerebral and cerebellar atrophy on MRI.

After the perinatal period, the characteristic combination of neurological symptoms becomes obvious at 3–6 months of age with hypokinesia, marked truncal hypotonia, frog-like posture, and a mask-like face. However, increased deep tendon reflexes, pyramidal tract signs, oculogyric crises, ptosis, and miosis differentiate this syndrome from neuromuscular disorders. Paroxysmal periods of generalized malaise with

lethargy, irritability, sweating, and drooling are life-threatening.

Some patients may not develop pyramidal tract signs, oculogyric crises, bouts of vegetative disturbances, or progressive extrapyramidal symptoms. In these patients dystonia and rigidity are obvious. From 2 to 5 years muscle tone increases progressively, and contractures, failure to thrive, and immobilization may develop.

In patients with predominant motor dysfunction, the first symptoms consist of dystonia and rigidity in infancy to early childhood. The dystonia begins in the lower limbs and spreads to generalized dystonia (Rondot and Ziegler, 1983; Rondot et al., 1992). Tremor is also observed in infancy (Rondot and Ziegler, 1983). One patient (Grattan-Smith et al., 2002) developed shaking movements at 2 months of age that started in the leg and spread to the head, tongue, and arms, and at 6 months of age it appeared as tremor. The limb tremor worsened with attempted movements and that of the tibialis anterior muscle showed a frequency of 4 Hz (Grattan-Smith et al., 2002). The deep tendon reflexes were brisk and the patient had a spastic paraplegia (Brautigam et al., 1999). However, plantar responses are flexor. In some cases, the intensity of the motor disorder is less pronounced in the morning or after a nap and more marked in the evening. However, this feature is not constant and cannot be considered an essential diagnostic criterion (Rondot et al., 1992). In these patients academic progress is normal. One case with a compound heterozygote developed extrapyramidal symptoms before 13 months of age and was able to walk independently and appeared to have spastic paraplegia (Furukawa et al., 2001).

An alternative presentation includes severe axial hypotonia, hypokinesia, dystonia, hypomimia, ptosis, and oculogyric crises (Brautigam et al., 1998). Miosis and postural hypotension are also observed (Hoffmann et al., 2003), as well as paroxysmal irritability, sweating, hypersalivation, pyramidal signs, and intellectual impairment. Moreover, these infants exhibit progressive encephalopathy with seizures and microcephalus (Grattan-Smith et al., 2002).

Disorders of pteridine metabolism and TH deficiency show a broad spectrum of movement abnormalities and variable clinical course (Givanniello et al., 2007).

Diagnosis

Decreased CSF levels of HVA and 3-methyoxy-4-hydroxyphenylglycol, together with normal pterin, CSF tyrosine, and 5-HIAA concentrations, are the diagnostic hallmarks of TH deficiency. Measurements of HVA, vanillylmandelic acid, or catecholamines in urine are not relevant for diagnosing TH deficiency (Wevers et al., 1999). The diagnosis should be considered in all children with unexplained hypokinesia and other extrapyramidal symptoms (Dionisi-Vici et al., 2000). For definitive diagnosis genetic testing is recommended.

Treatment

Patients with encephalopatly do not respond to levodopa.

In patients with predominance of dystonia, levodopa produces favorable and sustained effects (Rondot and Ziegler, 1983). Although abnormal movements or dyskinesia occur, they regress when the dosage is decreased (Rondot et al., 1992). However, treatment with levodopa in dystonic TH deficiency is often limited by the appearance of intolerable side-effects, mainly hyperkinesia and ballism (Hoffmann et al., 2003). In one child with severe axial hypotonia and ballistic movements a combination of selegiline hydrochloride with low-dose levodopa was effective, though levodopa monotherapy was unsuccessful (Ludecke et al., 1996).

Molecular biology

The severity of recessive TH deficiency depends on the loci of mutation. Mutation of TH gene was first detected by Knappskog et al. (1995) as a point mutation (Q138K) located on chromosome 11p15.5 in two siblings who had levodopa-responsive dystonia. The residual activity of TH was about 15% of the corresponding wild-type human TH. One patient with a severe phenotype had a homozygous point mutation (L205P), which had even lower activity of approximately 1.5% of wild-type human TH (Hoffmann et al., 2003). Three other patients (compound heterozygotes) for TH mutations (two brothers) (Furukawa et al., 2001) and one isolated patient (Brautigam et al., 1999) showed dystonia as the main symptom, which responded well to levodopa. Patients with a branch site mutation have a severe clinical phenotype (Janssen et al., 2000). Thirteen separate mutations have been identified, and suggest the percentage decrement of TH activity may involve the site of the genotype.

Pathophysiology

TH catalyzes the rate-limiting step in the biosynthesis of DA, norepinephrine, and epinephrine. Deficiency of TH results in symptoms caused by these catecholamine deficiencies. Grattan-Smith et al. (2002) proposed signs of DA deficiency as tremor, hypersensitivity to levodopa, oculogyric crises, akinesia,