D. 考察

1. 観察と生活指導による脳卒中様発作の誘発予防

1) 異常の早期発見

脳萎縮に伴う無呼吸発作、過呼吸発作、痙攣および不穏状態、不随意運動などがあったこれらの症状のうち無呼吸発作、過呼吸発作、痙攣は、呼吸停止に移行する可能性があり急を要する症状である。しかし、ミトコンドリア脳筋症末期は様々な症状を呈することからこれらの兆候に気づくのは難しい。

2) 残存機能の活用による不安の軽減と尊厳

日常生活の援助は残存している味覚、嗅覚、皮膚触覚による快と不快の反応を表情や仕草で判断し、不快でなく快の時間を多く持つようにした。触覚を利用しコミュニケーション手段として合図を使ったことは、盲・聾のためコミュニケーション手段を失い世の中と隔絶された状態に至った患者に対しての不安の軽減にも役立っていたものと考えられる。触覚による振動や気配などの情報しかない状態での孤立感のなかで生活している。マズローは人間が有するニードを生理的欲求、安全・安楽の欲求、愛情の欲求（社会的承認）、自尊心の欲求、自己実現欲求の5つの構成要素で説明し「すべての人間は、本来、より発展しようと自分の持てる力を発揮し、生きようとする存在動機である」[9] (1900)と、自己実現に向かう人間の方向性、欲求の階層説を理論づけている。つまり、人間の欲求は生存のための低次の生理的欲求から安全・安楽の欲求、愛情の欲求（社会的承認）、自尊の欲求そして自己実現の欲求まで階層を成しているというものである。生理的欲求と安全・安楽の欲求は、生命活動の中心的役割を営んでいることを考えると、盲・聾・痴呆のため意思疎通ができない患児に対する看護援助として最優先されなければならない。また、愛情の欲求（社会的承認）、自尊心の欲求、自己実現の欲求は、人間の成長や生活・人生を豊かにする人間存在や人間の尊厳に関わるものであり、表現の方法は違っても人は誰でもこれらの「基本的欲求」があり、これらの欲求は潜在していることを念頭におく必要があろう。看護援助に際して言葉で自分の気持ちを表現できない患者に代わって、習慣や好みを知っている母親からの情報を参考にしたことで、患者の日常性や好みに近づくことができ不安や恐怖の緩和につながったのではないかと考える。また、患児の不随意運動や不穏状態は人格そのものではなく症状の一つであることを認識した上で、残存機能を生かして個別的な援助を編成する技術と固有の生活をサポートする援助が必要である。

3) 母親(家族)の不安が軽減できる

(1)治療に対する憂鬱さ・混乱　受け入れ難さのサポート

ミトコンドリア脳筋症の進行を受容し難い母親の気持ちが推察されるため、看護師はこのような母親の気持ちを受け入れ母親の話に耳を傾ける関わりが重要である。母親は患者の不穏状態に使用される鎮静剤の使い方に不満を漏らしたが、母親にすればこれらの現実は受け入れ難いことであり、母親の深刻な気持ちを受け止め関わることが必要であろう。[10] (引用文献)

(2)社会サービスへの課題

療養型の転院先が見つからず精神病院の痴呆老人の病棟に入院したこと、そのため痴呆症状が出現した場合、入所者との年齢の違いから多くの施設が受け入れに躊躇している。痴呆老人の施設からの受け入れが可能だとしても、同世代の患者が入所している施設に入所させたいという当然の母親の気持ちにそった受け入れ施設を整備するが必要であろう。ミトコンドリア脳筋症の場合は小児慢性特定疾患事業の慢性特定疾患群に該当していないため18歳までは医療費扶助があるが、次のような場合には医療費扶助の対象ではない。ミトコンドリア脳筋症の脳卒中様発作で入院する場合(短期間入院と繰り返しの入院が多いため)、在宅療養の必要機器や消耗品など、18歳以上になってもミトコンドリア脳筋症は成人の慢性特定疾患研究事業の認定をされていない、筋・神経疾患で認定されたとしても入院期間が一か月以上でなければならない、若年性痴呆患者の介護保険適用がないことなどがある。これらのことより、ミトコンドリア脳筋症患者家族の経済的負担が大きいものと考えられるため、早急な小児慢性特定疾患事業の適用が必要とされる。

(3)遺伝病についての課題

母親はミトコンドリア脳筋症が遺伝性疾患であり自分自身の健康や長女の将来に対しても強い不安を持っていた。深川[11]は前述した遺伝性疾患である先天代謝異常の母親への心理的サポートについて「母親の精神反応はショック～遺伝性・病名の拒否～親である罪悪感～悲しみ～怒り～適応といったプロセスをたどる傾向がある。よって危機プロセスの経過の観察

と危機を乗り越える援助が必要である」と述べている。これらのことから、専門的な医療のカウンセリングが必要であり、患者・家族の不安や不満を吐露し、支えられる専門スタッフの育成が必要性である。ここでの専門的関わりとは武田祐子・数間恵子[12]が遺伝性腫瘍に対する心理的反応の受容への看護でのべている「患者や家族がその状況をどのように受け止めているのか傾聴し、あらゆる反応に対して受容的に対応していく」こととも重ねられる。つまり疾病の理解とコントロールができ、ソーシャルワークも含めて患児と家族の不安や葛藤に対処できる関わりであろう。

E. 結論

MELAS患者では、末期には失明、聴覚喪失、認知障害が起こる。介護としては、従来予測されていない精神的身体的に広範な社会的サポートが必要である。

F. 健康危険情報

特になし

G. 研究発表

論文発表

なし

学会発表

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H. 知的所有権の出願・取得状況（予定も含む。）なし

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

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
古賀靖敏	MELAS and L-arginine therapy.	Brain Dev.	26(7)	480	2004
	Noonan syndrome, moyamoya- like vascular changes, and antiphospholipid syndrome.	Pediatr Neurol.	31(5)	364-366	2004
	L-arginine improves the symptoms of stroke-like episodes in MELAS.	Neurology	64(4)	710-712	2005
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	MELASの新しい治療法－L-アルギニン	臨床検査	49(1)	83-88	2005

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Discussion

MELAS and L-arginine therapy

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Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is a maternally inherited multisystem mitochondrial disorder characterized by stroke-like episodes before 20 years of age [1]. Mitochondrial angiopathy, with degenerative changes in small arteries and arterioles, has been reported in many MELAS patients [2]; these blood vessels have been designated as strongly succinate dehydrogenase-reactive vessels (SSVs) [3]. However, the primary cause for stroke-like episodes in young MELAS patients—whether mitochondrial cytopathy, angiopathy, or both—remains controversial. Many therapeutic trials have been undertaken to cure mitochondrial disorders, but not for the acute stroke phase of MELAS. Based on a hypothesis that stroke-like episodes in MELAS are caused by segmental impairment of vasodilation in intracerebral arteries, L-arginine has been used for therapeutic trials in MELAS patients during the acute phase of stroke. We found that L-arginine therapy quickly decreased severity of stroke-like symptoms in MELAS, enhanced dynamics of microcirculation, and also reduced tissue injury from ischemia [4]. L-arginine is a potent vasodilator via endothelial function through nitric oxide (NO) production [5]. Cardioprotective effects of L-arginine and NO are associated with endothelial cell preservation [6], decreased neutrophil activation [7], improved coronary blood flow, and reduced free radical-mediated injury [8]. Although the molecular mechanism of L-arginine therapy in MELAS is not known, it is a potential new therapy for use at the acute phase of stroke-like episodes in MELAS.

In this paper, Dr Kubota and colleagues have performed a therapeutic trial to a 16-year-old girl with an acute phase of MELAS, using L-arginine infusion in order to improve the symptoms from stroke-like episodes. Among one out of five severe stroke-like episodes, L-arginine was added on the conventional steroid and glycerol therapy at the fifth episode. The authors described that L-arginine improved the symptoms much earlier than those without L-arginine infusion and shortened the duration of hospitalization. They measured the lactate level by MRS, which is a good indicator of the therapeutic effect. This is a case report describing a successful therapeutic result in MELAS. The finding will provide information about a new therapeutic approach to this currently incurable disease.

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Noonan Syndrome, Moyamoya-like Vascular Changes, and Antiphospholipid Syndrome

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This reports describes a 12-year-old Japanese female with Noonan syndrome who had antiphospholipid syndrome and moyamoya-like vascular changes. She presented choreic movements in her face and extremities. She manifested phenotypic features of Noonan syndrome with short stature, mental retardation, and a webbed neck. Magnetic resonance angiography revealed occlusion of bilateral internal carotid arteries and moyamoya-like vascular changes around the basal ganglion region. Pimozide completely resolved the patient's choreic movements. Tests for anticardiolipin antibody and lupus anticoagulant were positive. The patient has manifested no symptoms for 2 years with pimozide, aspirin, and growth hormone treatment, without further aggravation of moyamoya-like vascular changes. This article is the first report of Noonan syndrome with antiphospholipid syndrome and moyamoya-like vascular lesions.

Yamashita Y, Kusaga A, Koga Y, Nagamitsu S, Matsuishi T. Noonan syndrome, moyamoya-like vascular changes, and antiphospholipid syndrome. Pediatr Neurol 2004;31: 364-366.

Introduction

Noonan syndrome is characterized by a normal karyotype and clinical features that resemble Turner syndrome. An association between Noonan syndrome and moyamoya has been reported in only two cases [1,2]. In adults, the association between strokes and antiphospholipid antibodies has been established, but such an association with moyamoya disease has not been identified.

This report describes for the first time a first patient with a rare combination of Noonan syndrome, moyamoya-like vascular changes, and antiphospholipid syndrome.

Case Report

In June 2001, a 12-year-old female experienced difficulty in walking because of involuntary movement in the left upper and lower limbs. She was observed at Saga University Hospital. Cranial magnetic resonance imaging was normal. By the end of June, her symptoms improved. She was diagnosed as having antiphospholipid syndrome because of thrombocytopenia and positive anticardiolipin antibody. None of her family members had similar problems. The family did not return to the hospital because the patient was completely well. In February 2002, however, she had chorea again starting from the right hand and extending to the right foot. In April, her chorea extended to the left side and she began to speak less. The family saw a neurosurgeon, and cranial magnetic resonance angiography revealed moyamoya-like vascular changes. The patient was then referred to Kurume University Hospital for further evaluation and treatment.

On admission, her height was 120.8 cm (−5 SD) and her weight was 27 kg (−2 SD). She had multiple purpura lesions in her extremities. She manifested phenotypic features of Noonan syndrome, including a webbed and short neck, low posterior hair lines, low-set and abnormal auricles, and hyperterolism. She manifested no signs of lupus or secondary sexual development. The patient had no history of seizures, and no cardiac lesions were evident. There was no family history of thrombosis.

Neurologically, she was alert, but had dysarthria caused by facial chorea. Her chorea was more prominent in the right side upper and lower limbs with hypotonia. Her deep tendon reflexes were elevated, and the Babinski reflex was positive on the right side. Her full intelligence quotient (Wechsler Intelligence Scale for Children–third version [WISC-III]) was 53; verbal intelligence quotient 60, performance intelligence quotient 55. Her social performance was relatively good, and she was in a mainstream classroom.

Complete blood count revealed low platelets (90,000). Prothrombin time and activated partial thromboplastin time were mildly prolonged, and lupus anticoagulant 1.76 (normal <1.3), anticardiolipin antibody 100 (normal <10), anti-b2 glycoprotein, and antinuclear antibody were positive. Anti-Sm, anti-RNA, anti-SS-A/Ro, and anti-SS-B/La antibodies were negative. Protein C and S were normal. Her bone age was delayed, and growth hormone secretion was abnormal by both an L-arginine and glucagon loading test. Auditory brainstem response and electroencephalogram were normal. The cranial computed tomography revealed mild

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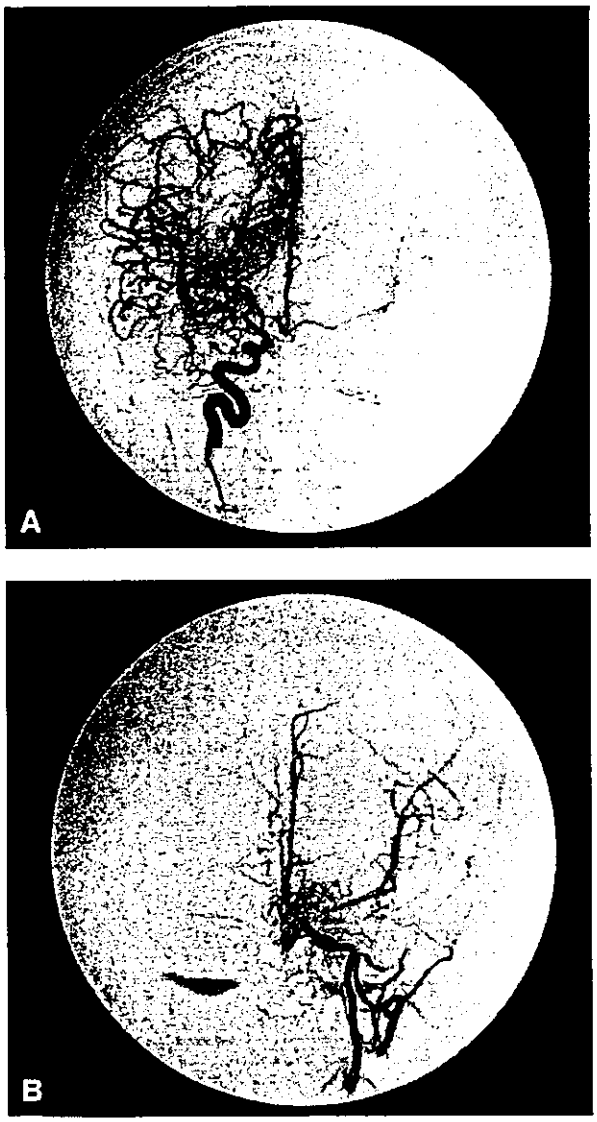

Figure 1. Cerebral angiogram: Right carotid angiogram indicated severe stenosis of internal carotid artery terminal lesion (A), and left carotid angiogram (B) demonstrated severe stenosis in the supraclinoid segment and collateral moyamoya-like vascular changes distal to the stenosis. The left internal carotid artery was narrower than the left side.

atrophy of the parietal lobe in the right hemisphere. The cranial magnetic resonance imaging revealed low-intensity areas in the basal ganglia on the right side. The cranial magnetic resonance angiography documented bilateral stenosis of the internal carotid arteries, which was more prominent on the right side, and moyamoya-like vascular changes in the bilateral basal ganglia and thalami region (Fig 1). Her chromosome count was normal (46 XX).

The patient was treated with a D2 antagonist, pimozide (1.5 mg/day), and her chorea completely resolved by the end of May. She has manifested no symptoms for 2 years with pimozide, aspirin (100 mg/day), and growth hormone treatment, with no further aggravation of moyamoya-like vascular changes.

Discussion

This patient was diagnosed with antiphospholipid syndrome because she had thrombocytopenia, positive anticardiolipin antibody, and lupus anticoagulant. These findings were repeatedly demonstrated at 10-month intervals. Furthermore, the patient manifested characteristic features of Noonan syndrome, including short stature, facial and neck abnormalities, mild mental retardation, and a normal karyotype. On magnetic resonance angiography, she also manifested moyamoya-like vascular changes in bilateral carotid arteries and basal ganglia. She was diagnosed with moyamoya disease because she had underlying Noonan syndrome and antiphospholipid syndrome.

Booth et al. have reported a 7-year-old female with an ischemic event in association with repeated elevation of anticardiolipin antibody [3]. They demonstrated bilateral moyamoya-like vascular changes. The patient was treated with warfarin for 5 months, followed by aspirin. Eleven months after the treatment, a marked improvement in blood flow with decreased stenosis of the left internal carotid was observed. They speculated that the development of moyamoya-like vascular changes might be secondary to initial thrombosis and stenosis of the basal cerebral vasculature with subsequent formation of collateral vessels. Takahashi et al. reported eight children with acute hemiplegia, with three being diagnosed as having infarctions due to moyamoya [4]. Anticardiolipin immunoglobulin G antibody was positive in three of the five with idiopathic infarction, but none with moyamoya disease, suggesting that the etiology of the infarct might be distinct from that in the patients with idiopathic infarction. In contrast, Bonduel et al. reported detecting prothrombotic disorders in 4 of 10 patients with moyamoya disease, suggesting its role in the pathogenesis of moyamoya [5]. Shoning et al. reported eight children who suffered from cerebrovascular ischemia or stroke in which antiphospholipid antibodies were detected. In two patients, stenoses of the basal cerebral arteries were present; a 5-year-old female with moyamoya-like vascular changes manifested improved circulation after treatment with aspirin and intravenous immunoglobulin, whereas a male patient required surgery for encephalo-duro-arterio-synangiosis [6].

There have been only two case reports of Noonan syndrome and moyamoya [1,2]. One of these patients had activated protein C resistance, which was thought to be coincidental. Antiphospholipid antibodies were not measured in these cases. Both patients were treated with aspirin and were responsive to nonsurgical therapy.

Encephalo-duro-arterio-synangiosis surgery was also considered for the patient in the present report; we decided not to pursue this route, however, because pimozide dramatically improved her symptoms and no further recurrence or progression of moyamoya-like vascular change occurred. In patients with moyamoya disease or moyamoya-like vascular changes, the possibility of antiphospholipid syndrome should be considered.

The authors gratefully acknowledge the assistance of Dr. Muneyuki Matsuo at the University of Saga, Department of Pediatrics.

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HIGH LIGHT

L-Arginine improves the symptoms of strokelike episodes in MELAS

Abstract—Based on the hypothesis that mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS) are caused by impaired vasodilation in an intracerebral artery, the authors evaluated the effects of administering L-arginine, a nitric oxide precursor. Patients were administered L-arginine intravenously at the acute phase or orally at the interictal phase. L-Arginine infusions significantly improved all strokelike symptoms, suggesting that oral administration within 30 minutes of a stroke significantly decreased frequency and severity of strokelike episodes.

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Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS) is a maternally inherited, multisystem mitochondrial disorder.[1] The primary cause of strokelike episodes in young patients with MELAS, whether mitochondrial cytopathy, angiopathy, or both, remains controversial. Based on the hypothesis that strokelike episodes in MELAS are caused by segmental impairment of vasodilation in intracerebral arteries, we administered L-arginine by IV administration during the acute phase of strokelike episodes and by oral administration during the interictal phase.

Methods. *Patients.* We studied 24 patients referred to the hospital with MELAS diagnosed according to clinical, muscle pathologic, and genetic studies and 72 healthy control subjects (table 1). Patients with congenital anomalies, sepsis, IV hyperalimentation, diabetes mellitus, cardiac failure, or a bedridden state were excluded from this study.

Study design. All patients or patients' parents gave written informed consent, and the L-arginine study protocol was approved (Kurume University IRB no. 9715). The study design was chosen because of patients' availability and finances did not permit a balanced, randomized design involving multiple centers. Our strokelike episodes fulfilled the criteria that patients have migraine headache, vomiting, convulsion, and transient blindness with brain image suggesting focal brain abnormality. Twenty-four patients with a total of 34 strokelike episodes took part in this study of L-arginine versus placebo, following a previously described protocol.[2] The severity of a strokelike attack (convulsion, cortical blindness, hemiparesis, or abnormality in brain images associated with headache and vomiting) was similar when either L-arginine or placebo was administered.

Six patients were treated by oral administration of L-arginine to prevent strokelike episodes. Four to 24 g of L-arginine (Arugi U, Ajinomoto Pharma; 0.15 to 0.3 g/kg/d) were given orally for 18 months. Patients were monitored clinically and biochemically as described previously once every 2 weeks. When patients were admitted to the hospital with a strokelike episode, the following symptoms were scored: headache (present: 1, none: 0), vomiting (present: 1, none: 0), teichopsia (present: 1, none: 0), convulsion (present: 1, none: 0) and hemiparesis (present: 1, none; 0).[3] For each admission during the study period, these scores were summed as the severity score for the stroke. Frequency of admission was taken to be the frequency of strokelike episodes. Severity and frequency were related to time as number and month and were compared between periods 18 months before and after oral administration of L-arginine in the same patient.

Analysis of amino acids, asymmetric dimethylarginine (ADMA),[4] nitric oxide (NOx),[5] cyclic guanosine monophosphate (cGMP)[6] were measured using described methods.

Analysis. Plasma concentrations of amino acids, NOx, and ADMA in patients in the acute or interictal phase of MELAS were compared with those in controls using unpaired *t* tests, with Bonferroni corrections for outlying values. Concentrations of L-arginine, L-citrulline, NOx, ADMA, and cGMP in plasma obtained before, 30 minutes after, and 24 hours after L-arginine infusion were compared with those in controls using paired *t* tests. Statistical analysis of clinical improvement was performed using Fisher's exact test. Frequency and severity of strokelike episodes in six patients with MELAS after long-term oral L-arginine supplementation were compared with those in the same patients without supplementation using a nonparametric Mann–Whitney *U* test. All data are presented as means ± SD. p Values of 0.05 or less were considered to indicate significance.

Results. Baseline characteristics of the 24 patients and 72 controls are shown in table 1. Mean plasma concentrations of L-arginine and L-citrulline were lower in both acute (L-arginine: 47 ± 13 μmol/L; L-citrulline: 23 ± 10 μmol/L) ($p < 0.01$) and interictal phases (L-arginine: 84 ± 26 μmol/L; L-citrulline: 26 ± 10 μmol/L) ($p < 0.01$) of MELAS than in controls (L-arginine: 108 ± 28 μmol/L; L-citrulline: 35 ± 9 μmol/L). Concentrations of L-arginine in the acute phase were also significantly lower than in the interictal phase, whereas those of L-citrulline did not show a significant phase-related change. NOx concentrations were lower in the acute phase (24 ± 10 μmol/L) ($p < 0.01$) of MELAS than in controls (45 ± 30 μmol/L), whereas in the interictal phase (91 ± 44 μmol/L) ($p < 0.01$), they were higher than in controls. Conversely, concentrations of ADMA did not significantly differ between controls and acute phase, although the ADMA/L-arginine ratio was higher in the acute phase (0.011 ± 0.004) ($p < 0.01$) than in the controls (0.005 ± 0.001) or in the interictal phase (0.005 ± 0.001).

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Table 1 Baseline characteristics of the patients with MELAS and controls

Variable	Patients with MELAS (n = 24)	Controls (n = 72)
Age, y (range)	19.6 ± 12.5 (8.2–30.3)	21.5 ± 10.4 (4.3–35.4)
Gender, M/F	8/16	27/45
BMI	17.8 ± 3.6*	20.4 ± 2.3
Height	−2.2 ± 0.8*	0.2 ± 0.9
Alanine, μmol/L plasma	514 ± 164*	406 ± 121
Pyruvate, μmol/L	0.22 ± 0.06*	0.08 ± 0.05
Lactate, μmol/L	4.5 ± 1.8*	0.8 ± 0.2
L/P ratio	19.8 ± 2.9*	10.5 ± 1.8
Total cholesterol	139 ± 27	135 ± 38
LDL cholesterol	13.8 ± 3.7	14.6 ± 5.9
A3243G in muscle, %	68 ± 16	ND
Ragged-red fibers in muscle, %	3.6 ± 1.9	ND

Plus-minus values are means ± SD.
* $p < 0.05$ compared with controls.

MELAS = mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes; BMI = body mass index; L/P = lactate pyruvate; LDL = low density lipoprotein; ND = not detectable.

Symptoms and biochemical measurements after L-arginine therapy in the acute phase of strokelike episodes in MELAS are shown in table 2 and the figure. After administration of L-arginine, all symptoms suggesting stroke dramatically improved. No adverse effects occurred, except headache, when L-arginine was infused too rapidly in two patients. With treatment, concentrations of lactate and pyruvate, L-arginine, L-citrulline, NOx, cGMP, and ADMA returned to interictal-phase concentrations within 24 hours.

After oral L-arginine supplementation, the frequency and severity of symptoms caused by the stroke had decreased dramatically. Frequency of strokelike episodes after treatment (0.09 ± 0.09) ($p < 0.05$) decreased compared with before supplementation (0.78 ± 0.42). The severity score after treatment (0.17 ± 0.18) ($p < 0.05$) was also lower than before supplementation (2.04 ± 0.34). After L-arginine supplementation, no patient with MELAS had a major strokelike attack, including hemiconvulsion or hemiparesis, but only headache or teichopsia. Plasma concentrations of L-arginine in patients with MELAS ranged from 82 to 120 μmol/L (mean ± SD 92 ± 17 μmol/L) after initiation of L-arginine supplementation.

Discussion. L-Arginine, which plays an important role in endothelium-dependent vascular relaxation, was significantly lower in both the acute and interictal phases of MELAS than in control subjects. Why plasma L-arginine is decreased in the acute phase of MELAS remains to be elucidated. We analyzed the

Table 2 Effects of L-arginine on the clinical symptoms in acute phase of MELAS

	Time after administration			
	Before	15 min	30 min	24 h
Headache (improvement of score from 3/2 to 1/0)				
L-Arginine	0/22	2/22	18/22*	21/22*
Placebo†	0/12	0/12	1/12	1/12
Clinical disability (improvement of score from 3/2 to 1/0)				
L-Arginine	0/22	3/22	16/22*	20/22*
Placebo†	0/12	0/12	1/12	1/12
Nausea				
L-Arginine	0/22	2/22	15/22*	22/22*
Placebo†	0/12	0/12	0/12	1/12
Vomiting				
L-Arginine	0/22	3/22	18/22*	22/22*
Placebo†	0/12	0/12	0/12	1/12
Hemi-blindness (transient)				
L-Arginine	0/7	2/7	4/7*	7/7*
Placebo†	0/4	0/4	1/4	1/4
Teichopsia				
L-Arginine	0/22	0/22	8/22*	19/22*
Placebo†	0/12	0/12	0/12	0/12

Numbers indicate the number of occasions when improvement was seen relative to the total number of episodes.
* $p < 0.05$ by Fisher's exact test.
† 5% dextrose (0.5 g/kg/dose) in eight episodes, and D-arginine in four episodes.

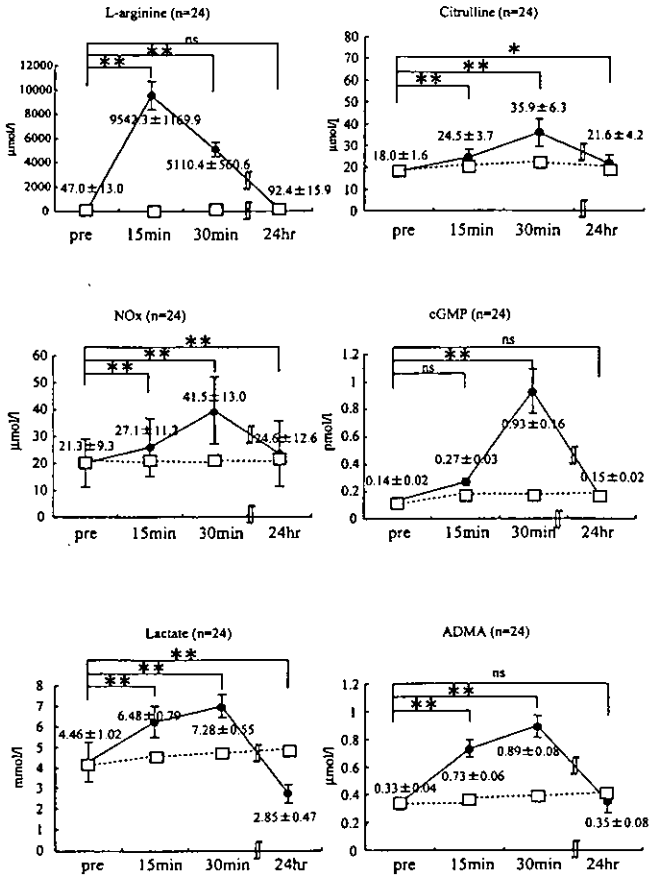

*Figure. Plasma concentrations of L-arginine, L-citrulline, nitric oxide (NOx), cyclic guanosine monophosphate (cGMP), lactate, and asymmetric dimethylarginine (ADMA) before and after L-arginine therapy in the acute phase of strokelike episodes in MELAS. Data represent mean ± SD (μmol/L) (n = 24). *p < 0.05; **p < 0.01 vs values before L-arginine therapy. ns = not significant. Filled circles show biochemical analysis after L-arginine therapy. Open squares show biochemical analysis after administration of placebo.*

correlation in all amino acids and found that the decrease of L-arginine in the acute phase is not influenced by urea cycle activities but may be caused by endothelial dysfunction (data not shown). A low L-arginine concentration and a relatively high ADMA concentration may predispose to strokelike episodes in MELAS. Impairment of endothelial function associated with relatively increased ADMA concentrations is reversed by IV L-arginine.[7] Consistent with these data, L-arginine infusion improved the ischemic process during the acute phase of MELAS.

Focal cerebral hyperemia has been reported in MELAS.[8] Although the underlying mechanisms are incompletely understood, hyperemia is thought to reflect vasodilation caused by local metabolic acidosis in the area of the infarct or by the foci of periodic epileptiform discharge.[9] Because the above studies were performed several days or several weeks after the onset of a strokelike episode, secondarily induced NOx production generated by inducible NOx synthase in the injured region may alter evidence of the primary pathophysiologic abnormality. In an analysis of SPECT findings in young patients with MELAS at a very early stage of strokelike episodes (within 3 hours after onset), we found hypoperfusion in the region affected by the strokelike episode. We cannot explain conclusively why our findings differ from those reported by neurologists treating adults. If the sites of angiopathy in MELAS most likely include small cerebral arteries, arterioles, and capillaries, small infarcts would be expected rather than the large confluent region of infarction described in many reports of MELAS. L-Arginine is an important precursor of NOx, which may reduce ischemic damage in the acute phase of focal brain ischemia by increasing microcirculation in the cerebral blood flow. The symptoms improved earliest, and magnetic resonance spectroscopy abnormality was minimal when L-arginine was given during the acute phase of strokelike episodes in MELAS.[10]

We evaluated the effects of oral L-arginine supplementation on long-term occurrence of strokelike episodes. The frequency and severity of clinical symptoms of strokelike episodes decreased without serious adverse effects. Prophylactically treated patients with MELAS have not had major strokelike attacks such as hemiconvulsion and hemiparesis. Headache and teichopsia have occurred.

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Title: A new sequence variant in mitochondrial DNA associated with high penetrance of Russian Leber hereditary optic neuropathy

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Abstract

We have analyzed mitochondrial DNA sequence in 15 Russian LHON patients and found the new mtDNA sequence variant in one family (2 patients) who showed 100% penetrance of the disease in men. This family has a T14484C primary mutation, and 4 secondary mutations (T4216C, G 13708A, G 15812A, G15257A), which belong to the European haplogroup J. The new sequence variant of A9016G in the ATPase 6 gene changed highly conserved amino acid of isoleucine to valine, has not been found in the rest of 13 LHON patients and controls. This novel sequence variant may contribute to the 100% penetration of LHON disorder in men of this family.

Key words: LHON, point mutation, penetrance, mitochondrial DNA

Introduction

Leber Hereditary Optic Neuropathy (LHON [MIM 535000]) is a maternally inherited genetic disorder associated with point mutations in the mitochondrial DNA (mtDNA) that cause blindness in young adults (Wallace et al., 1988; Howell. et al., 2003). It is characterized by acute or subacute, progressive, and bilateral loss of central vision due to focal degeneration of the retinal ganglion cell layer and optic nerve. Common primary LHON mutations are G3460A, G11778A, and T14484C not in association with each other, heteroplasmic and are absent among control are necessary for expression of the disease but not sufficient (Man et al., 2002). Secondary/intermediate LHON mutations showed different frequency pattern among the three classes of above common primary mutations in positive patients and controls, usually homoplasmic, and may contribute to the LHON by increasing the probability of expressing the phenotype (Torroni et al., 1997).

Patients and methods.

Patients.

Fifteen patients from 13 unrelated Russian families were fulfilled the clinical criteria of LHON (Wallace et al., 2001), however, patients 8, 9, 11, 12, 13, 14 and 15 have no family history. Three women and twelve men from 10 to 56 years old with mean age of onset about 22.3 years. The research followed the tenets of the Declaration of Helsinki. Informed consent

was obtained from the patients after explanation of the nature and possible consequences of the study. DNA was taken and studied for the various mtDNA mutations.

One family which has the highest penetrance among our 13 unrelated families is shown in Figure 1. The proband is a 35-year-old Caucasian man, who suffered from a rapid bilateral painless loss of central vision. The loss of vision began at the age of 18 years, and at the same period, the patient was admitted to the hospital with diabetic coma due to insufficient insuline production. He had a 6-month interval between the blindness in both eyes which was not caused by diabetic retinopathy. The visual acuity is 0.02-0.03 with the central scotomas. Bilateral loss of central vision was also detected in patient's mother, a 56-year-old woman, when she was 47 years old. She had two brothers and two uncles who were completely blind from their youth, showing 100% penetrance of LHON disease in men of maternal lineage. There is no family history of diabetes mellitus, deafness or neuromuscular disorders except blindness.

Sequencing analysis.

Total DNA was extracted from white blood cells using the standard method (King et al., 1992). The complete mitochondrial genome was amplified by long-PCR method in 7 overlapping fragments using 14 primers (Table1). The PCR conditions were: first, one cycle of 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 57 °C for 1 min, 72 °C for 3 min, and finally, one cycle of 72 °C for 5 min followed by cooling to 4 °C. The quality and quantity of

template DNA were determined by 1% agarose gel electrophoresis. ExoSap-IT (USB Corporation, Ohio USA) utilizes two hydrolytic enzymes Exonuclease I and Shrimp Alkaline Phosphatase together in a specially formulated buffer, to remove unincorporated dNTPs and primers. The ExoSap-IT was added directly to the PCR product. After treatment, ExoSap-IT was inactivated simply by heating to 80 °C for 15 minutes. Using 40 forward primers (Table 2) and CEQ Dye Terminating Cycle Sequencing Kit (Beckman Coulter, Inc., Fullerton, CA), sequence reaction was performed as following: first, one cycle of 96 °C for 5 min, followed by 30 cycles of 96 °C for 20 sec, 50 °C for 20 sec, 60 °C for 3 min, and finally, one cycle of 60 °C for 5 min followed by cooling to 4 °C. The sequences were assembled in a contig using the program DNASIS Pro (Hitachi Software Engineering Co, Ltd, Tokyo, Japan) and the resulting contig was aligned to the Cambridge sequence (Anderson et al., 1981; Andrews et al., 1999).

PCR-RFLP analysis.

To confirm the heteroplasmic condition in primary and secondary mutation reported in LHON, or novel sequence variant, we performed the PCR-RFLP analysis using suitable sets of primers and restriction enzymes (Brown et al., 1995; Brown et al., 2001). Conditions for all PCR amplifications were as follows: first, one cycle of 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55.5 °C for 1 min, 72 °C for 45 sec, and finally, one cycle of 72 °C for 5 min followed by cooling to 4 °C. Diagnostic restriction endonuclease digestions were resolved by 12% polyacrylamide gel electrophoresis, and the DNA fragments were identified by ethidium

bromide.

For new A9016G sequence variant, we designed a set of primers as following: forward from np8833 to np8852 and reverse from np9140 to np9121 (5'-3').This fragment contains a single restriction site for HpyCH4IV in the presence of mutation. To eliminate partial enzymatic digestion, all restriction enzyme reactions were carried out overnight, followed by the addition of 10 units of enzyme and 2 additional hours of incubation. Percentage of heteroplasmy was quantitated by one-dimensional densitometry using Image Quant software version 4.1 (Molecular Dynamics, Sunnyvale, CA, USA).

Results.

All nucleotide changes found in our study are shown in Table 3. Seven patients from 5 unrelated families have one of the primary mutations. Two unrelated patients (patients 1 and 7) have a G11778A mutation. One of them (patient 7: haplogroup J) has two secondary mutations of T4216C and G13708A, however, the other (patient 1: haplogroup W) has no secondary mutation. Three patients (2 families) have a G3460A mutation, one family (patients 4 and 5: haplogroup X) has no secondary mutations, and the other (patient 6: haplogroup T1) has a T4216C and A4917G mutations. Patients 2 and 3 (mother and son) belong to haplogroup J and have a primary mutation of T14484C and secondary mutations of a T4216C, G13708A, G15257A, and G15812A. Eight patients from 8 families (patients from 8 to 15) have no known

primary mutation in their mtDNA, however 3 patients (patients 8, 12, and 13) from this group have one of the secondary mutations of G13708A or A4917G. Remaining 5 patients have none of pathogenic mutations reported before. Moreover, we have found a novel sequence variant of A9016G in the ATPase 6 gene in mother and son (patients 2 and 3) who have a primary mutation of T14484C (Figure 2).

Among 13 unrelated families from Russian LHON, distribution of mtDNA haplogroups were as follows: 23% for haplogroup H (n=3) ; 23% for haplogroup J (n=3) ; 15.4 % for haplogroup X (n=2) ;15.4 % for haplogroup T (n=2) ; 7.7 % for haplogroup U2 (n=1) ; and 7.7 % for haplogroup M (n=1).

Discussion.

LHON is characterized by incomplete penetrance and men are preferentially affected (~68%) when the primary mutation of T14484C exist (Wallace et al., 2001). Since our family (patients 2 and 3), who showed 100% penetrance of the disease in men in three generations, has the nucleotide changes at C5633T, A11251G, A12612G, and C15452A in addition to primary mutation of T14484C in the homoplasmic condition, (Herrnstadt et al., 2002) they are constituent of European mtDNA haplogroup J background which is present in European population at a frequency of 9 %. The other factors such as smoking, alcohol excess, diet, psychological stress, exposure to toxins, head trauma have been among the epidemiologic

factors suspected of increasing the penetrance of LHON (Tsao et al., 1999). However in our family, there are no obvious risk factors to increase the penetrance of this disorder. We found the novel A9016G sequence variant in both mother and son in this family. This sequence variant was heteroplasmic and results in a substitution of isoleucine for valine at a highly conserved residue in the ATPase 6 polypeptide (Figure 3). Hence, this is likely to be pathogenic mutation according to above information (Riordan-Eva and Harding ,1995; Brown et al., 2002). This sequence variant has not been reported in the literature, MITOMAP and mtSNP database, and has not been found in 13 Russian LHON patients and in 30 Japanese individuals. Though a number of studies have failed to make the important distinction between frank pathogenic mtDNA mutations and haplogroup-associated polymorphism (Chagnon et al., 1999; Lin et al., 1992), this A9016G sequence variant may increase the penetrance of the disease in LHON, when it is associated with the primary mutation of T14484C and secondary mutations of a T4216C, G13708A, G15257A, and G15812A. In addition, this family has the unique combination of the following polymorphisms of A9494G, G11718A, A15662G, and C16193T which are reported in the literature, however, they are not present in the rest of 12 families in this study.

We could not perform further biochemical analysis in this index family, because specimens were not available. Thirty-seven to fifty percent of LHON having T14484C primary mutation are reported to be recovered within 16 months interval (Wallace DC et al., 2001, Web