

brain. However, neither diffuse neurofibrillary tangles nor calcification of vessels can be detected by CT, MRI, single photon emission computed tomography (SPECT) and positron emission tomography (PET). Only CT can detect punctate and patchy calcifications in the basal ganglia and the cerebellum, which appears similar to the tip of an iceberg on radiological images of the brain. Recently, MRI has been more frequently carried out than CT to examine the brain. However, the sensitivity of CT has increased in recent years compared with that in the 1980s. The elderly population has rapidly increased in recent years.

In the present study, we investigated the frequency of calcification in the basal ganglia and dentate nuclei in the cerebellum of patients who underwent CT in two representative university hospitals in Japan over period of 1 year. We aimed to show the existence of more patients with Fahr's disease and DNTC, respectively, by an up-to-date CT study in Japan.

## Methods

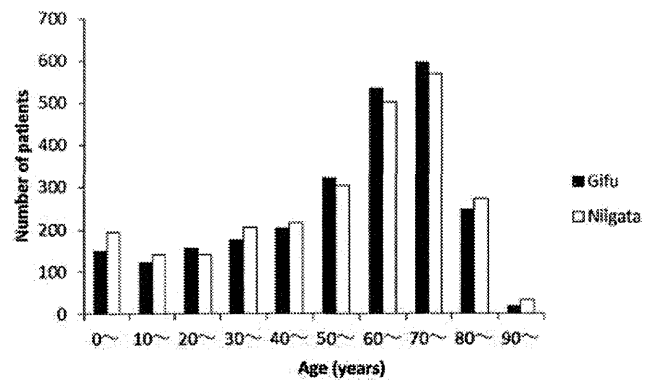
### *Patients and study area*

The study was carried out at two university hospitals: Gifu University Hospital (Gifu UH) in Gifu City and Niigata University Hospital (Niigata UH) in Niigata City. Gifu, a city with a population of 420,000, 270 km west of Tokyo, is situated around mountains. Niigata, a city of 800,000, 250 km north of Tokyo, faces the Japan Sea. The percentage of older adults (aged over 65 years) is the same (23%) in both cities. Both university hospitals had almost the same numbers of outpatients (between 1200 and 1500) per day.

The numbers of patients who underwent CT between October 2009 and September 2010 were almost the same: 2526 in Gifu UH and 2573 in Niigata UH. The number of older adults was 1163 in Gifu UH and that in Niigata UH was 1042. The numbers of patients at various decades of ages in both hospitals are shown in Figure 1. Both hospitals had almost the same numbers of patients who underwent CT.

CT was carried out by using Light Speed Ultra (GE Healthcare, Tokyo, Japan), Light Speed Ultra 16 (GE Healthcare) and Brilliance 64 (PHILIPS, Tokyo, Japan) in Gifu UH, and Toshiba Aquilion 64 (Toshiba, Tokyo, Japan) in Niigata UH with a 5-mm slice width, 80–100 of window width, 30–40 window mean. However, the sensitivity of CT did not differ significantly among these machines, at least for the detection of calcification.

The CT images were evaluated by the same three experienced neurologists. The study was approved by both the Ethics Committee of Gifu University, Graduate School of Medicine and the Ethics Committee of Niigata University, Graduate School of Medicine.



**Figure 1** Numbers of patients who underwent computed tomography at various decades of age in Gifu University Hospital (■) and Niigata University Hospital (□).

### *Inclusion criteria*

The criteria of calcification in the brain were as follows:

- 1 High-density areas appeared at the globus pallidus and/or dentate nucleus in the cerebellum on plain CT.
- 2 The high-density areas were more than 13 HU than the surrounding brain tissues.<sup>12</sup>
- 3 Hematoma, tumors, foreign bodies and other identified high-density areas were excluded.
- 4 The size and extent of lesions met the following definitions:
  - a Punctate lesions: having a maximum diameter of less than 10 mm or being sporadic.
  - b Patchy lesions: having a maximum diameter of 10 mm or more.

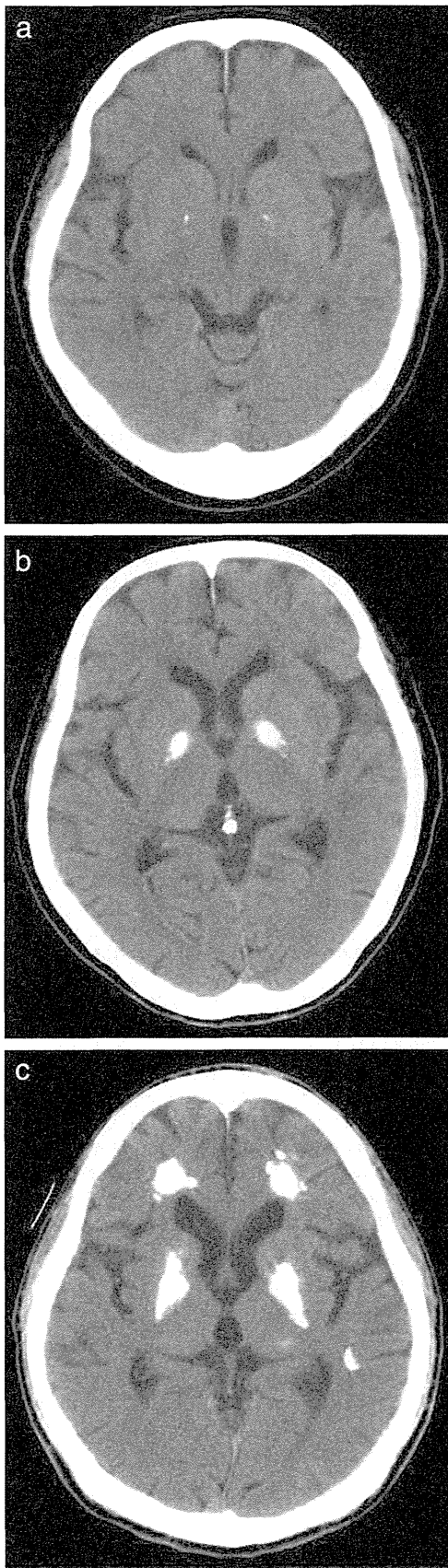
Representative CT images of patients with calcification are shown in Figure 2. The definition of Fahr's disease remains controversial. However, here it is defined as an idiopathic, non-arteriosclerotic calcification associated with cerebral vessels, particularly those in the basal ganglia, dentate nucleus, cerebral and cerebellar white matter.

### *Statistical analysis*

The patients were classified into two groups: the younger (younger than 65 years-of-age) and elderly (65 years-of-age or older) groups, on the basis of age at the time of CT. The  $\chi^2$ -test was carried out to analyze the relationship between the frequency of calcification and age. All analyses were carried out with Microsoft Excel (Microsoft Corp, Redmond, WA, USA). The level of significance was  $P < 0.01$ .

## Results

Among the 2526 patients in Gifu UH, 435 showed punctate calcification (17.2%) and 32 showed patchy



**Figure 2** Representative computed tomography images of brain with calcification. (a) Punctate lesions. (b,c) Patchy lesions. (c) A patient with Fahr's disease.

calcification (1.3%) in the globus pallidus (Table 1). Among the 2573 patients in Niigata UH, in contrast, 530 showed punctate calcification (20.6%) and 50 showed patchy calcification (1.9%) in the globus pallidus. Among the elderly patients, 24 (2.1%) in Gifu UH and 34 (3.1%) in Niigata UH showed patchy calcification. However, no significant differences were observed between the two university hospitals.

First, we focused on the age distribution of punctate lesions. The number of patients with such lesions was high in the elderly (Fig. 3). The frequency of punctate calcification was higher in the elderly group than in the younger group, but no significant difference was observed between Gifu UH and Niigata UH (Fig. 4).

Second, we focused on the age distribution of patchy lesions. The number of patients with such lesions was high in the elderly group. The frequency of patchy calcification was significantly higher in the elderly group than in the younger group in both Gifu UH and Niigata UH (Fig. 4), which showed a statistically higher significant difference in Niigata UH ( $P < 0.0005$ ) than in Gifu UH ( $P < 0.001$ ).

Calcification of the dentate nucleus in the cerebellum was observed in seven patients in Gifu UH and four patients in Niigata UH. All of these patients except one also showed patchy calcification of the basal ganglia. One exceptional elderly patient showed only punctate calcification, which might have been vascular in origin. The total number of calcifications in the dentate nucleus in the cerebellum was much smaller than expected. The etiologies were determined to be Fahr's disease and leukodystrophy in Gifu UH and secondary hypoparathyroidism in Niigata UH in the younger group. Five of the seven patients in Gifu UH and three of the four patients in Niigata UH were elderly. We were unable to obtain clinical information in detail, such as whether the elderly patients with calcification in both the basal ganglia and dentate nuclei in the cerebellum suffered from dementia. Calcification in these patients was incidentally found on their CT images taken because of to a head injury.

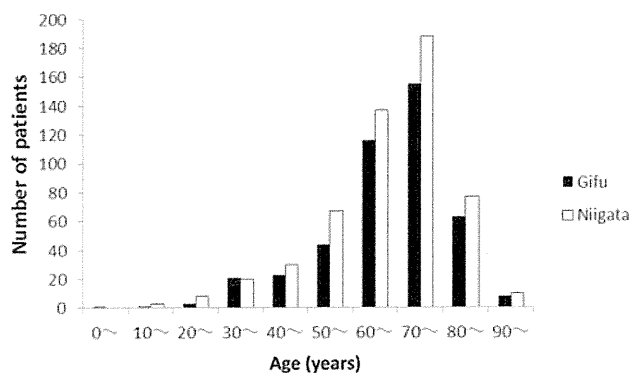
## Discussion

Calcification of the cerebellar dentate nucleus and/or basal ganglia is detected at rates of 40–72% of autopsy cases, and microscopic calcification in these areas exists more frequently.<sup>3</sup> Chemical analysis showed that calcium is the major element of calcification among minerals, and calcium hydroxylapatite ( $\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$ ) is a major component. Hydroxylapatite can be found in the teeth and bones in the human body. The other components are zinc, iron and magnesium. CT enables the detection of calcifications as high-density areas when the amount of components surpasses a certain limit.<sup>13</sup>

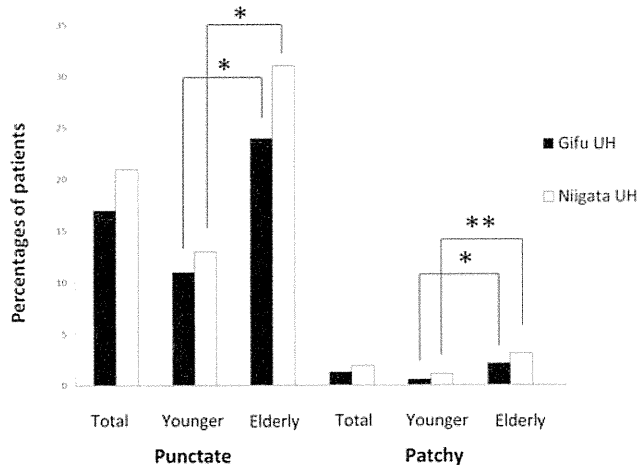
**Table 1** Numbers and percentages of patients with punctate and patchy calcification

|            | All         | Younger    |           | Elderly   |           |
|------------|-------------|------------|-----------|-----------|-----------|
|            |             | Men        | Women     | Men       | Women     |
| Gifu UH    |             |            |           |           |           |
| Total      | 2526        | 1363 (54%) | 613 (45%) | 591 (51%) | 572 (49%) |
| Punctate   | 435 (17.2%) | 151 (11%)  | 84        | 117       | 167       |
| Patchy     | 32 (1.3%)   | 8 (0.6%)   | 6         | 7         | 17        |
| Niigata UH |             |            |           |           |           |
| Total      | 2573        | 1531 (59%) | 764 (50%) | 516 (50%) | 526 (50%) |
| Punctate   | 530 (20.6%) | 190 (13%)  | 119       | 139       | 201       |
| Patchy     | 50 (1.9%)   | 16 (1.1%)  | 8         | 12        | 22        |

UH, University Hospital.



**Figure 3** Numbers of patients with punctate calcification detected on computed tomography images at various decades of age in Gifu University Hospital (■) and Niigata University Hospital (□).



**Figure 4** Percentages of patients with punctate and patchy calcification detected on computed tomography images of younger and elderly groups. The  $\chi^2$ -test was carried out to analyze the relationship between the frequency of calcification and age. \* $P < 0.001$ , \*\* $P < 0.0005$ . The frequency of punctate calcification in the elderly group was significantly higher than that in the young group. The statistical significance of the difference was observed to be higher in Niigata University Hospital (\*\* $P < 0.0005$ ) than in Gifu University Hospital (\* $P < 0.001$ ).

The mechanism underlying calcification remains unclear. The endothelial cells of capillaries and arterioles or pericytes, which control endothelial cells, are assumed to be damaged first for some reason. Subsequently, vascular permeability increases and the plasma protein leaks into the extravascular space. Deposits of electron-dense calcium bodies were found in the capillary walls and parenchyma by electron microscopy,<sup>14</sup> and were referred to as false calcification. Finally, the accumulation of calcium results in calcification in the lesion.<sup>2</sup>

The reason why the basal ganglia is vulnerable to calcium deposition also remains to be elucidated.

Considering the vascular structure and blood flow, the region is susceptible to oxygen depletion. The area tends to be a target for the deposition of minerals. Calcification easily occurs in various diseases and with age.

The present study showed that the frequencies of calcification, approximately 20% and 1–2% in punctate lesions and patchy lesions, respectively, in the basal ganglia were higher than those previously reported (0.3–10% by CT in the 1980s). This is assumed to be a result of the increase in the elderly population and the higher sensitivity of recent CT machines than before. Those frequencies might increase up to those of calcification seen in autopsy (40–72%). The frequency of punctate and patchy lesions increased with age. Among patients aged over 65 years, 24 (2.1%) and 34 (3.1%) showed patchy lesions in Gifu UH and Niigata UH, respectively. In contrast, the frequency of calcification of the dentate nucleus in the cerebellum was much lower than expected.

In autopsy, more than 10 cases of DNTC were reported in areas near Gifu, whereas no cases of DNTC were reported in areas around Niigata. Initially, we expected a higher frequency of calcification in Gifu areas than in Niigata areas; however, the number of patients with calcification, particularly patchy calcification, seems to be higher in Niigata areas than in Gifu areas, but no statistically significant regional difference was detected.

In the present study, we could not examine the clinical symptoms and signs in detail in the patients with calcification without permission of patients, following the regulation of ethics committees. The elderly patients with both patchy calcification in the basal ganglia and in the dentate nucleus in the cerebellum might have DNTC or subclinical DNTC. Unfortunately, we have no suitable equipment for the detection of diffuse neurofibrillary tangles or calcification of small vessels. Although MRI is widely used for the study of the brain, CT is necessary for the detection of calcification in the basal ganglia and the dentate nucleus in the cerebellum.

The frequency of calcification in the basal ganglia was higher, and that in the dentate nucleus was lower than we expected. We detected a new patient who might suffer from Fahr's disease by searching radiological reports in a hospital. Unfortunately in the present study, however, there was only one patient with Fahr's disease, whom we had already diagnosed in Gifu UH before this study. Four patients in Gifu and three patients in Niigata with calcification in both the basal ganglia and the dentate nuclei in the cerebellum were considered to possibly have DNTC. However, a more sensitive technique for the detection of calcification in the small

vessels and/or diffuse neurofibrillary tangles should be developed in the future for precise diagnosis.

## Acknowledgments

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## Disclosure statement

No potential conflicts of interest are disclosed.

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# 希少神経難病ファール病3例の患者と家族のインタビューから得られたもの

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## はじめに

ファール病(特発性大脳基底核・小脳歯状核石灰化症)の名称はドイツの病理学者 Theodor Fahr (1877～1945)に由来する。ファール病の原因はいまだに不明であり、ファール病の統一された診断基準もいまだない。ファール病の一般概念として、①脳内の大脳基底核や小脳歯状核に著しい石灰化をみとめる、②石灰化は両側対称性である、③副甲状腺機能異常症、ミトコンドリア脳筋症、原因の明らかな先天性代謝異常症などをみとめない、すなわち特発性であることがあげられる。

また近年、主に初老期に、脳内の石灰化とともに、びまん性の神経原線維変化をきたす疾患として小阪・柴山病

が少なからず存在することが見出され、時としてファール病との鑑別が困難である。ファール病は家族性の症例も少なからず存在し、またその発見の経緯も、若年者において外傷などの機会に頭部CT検査がなされ、無症状の状態で見られることが多い<sup>1)2)</sup>。そのため、若年者の患者やその家族は、その診断はまさに“青天の霹靂”で、医師から「原因不明で、進行性、治療もない」といった説明は“奈落の底に突き落とされた”思いであったと語っている。しかし、幸い、2010年、厚生労働省の後押しで難治性疾患克服研究事業の1つにファール病が取り上げられた。われわれは典型的な3症例の患者本人や家族と面談を行う機会を得たが、患者や家族の抱える課題を明らかにし、今後の医療体制の確立、患者と家族の心理的支援に役立てたいと考えた。

## 目的

ファール病と診断された患者、家族

に、診断されるまでの経緯、これまでに困ったことなどをインタビューし、ファール病と診断された患者や家族の療養における課題を明らかにする。それをもとに患者や家族の生活の質(QOL)の向上を目指し、患者、家族を支援する医療体制の向上と地域保健・医療・福祉の向上に役立てる。

## 研究の方法

### 1) 対象

臨床的また画像検査からファール病と診断された若年成人者(40歳以下)の典型的な症例患者またはその家族。

### 2) 面談方法

外来診察後、外来医師が患者より承諾を得たうえでインタビューを行った。面接は半構成的に、1回1時間以内を目安とし、個室で行った。

### 3) インタビューの内容

①病気が見つかった経緯、病名告知の

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表1 ●患者・家族の属性

| 対象番号    | 属性 | 年齢<br>(歳) | 患者<br>性別 | 患者年齢<br>(歳) | 発症<br>年齢(歳) | 患者<br>職業 | 患者<br>結婚 | 外来受診<br>回数 | 面談<br>時間 |
|---------|----|-----------|----------|-------------|-------------|----------|----------|------------|----------|
| No.1    | 本人 | 30        | 女性       | 30          | 18          | 事務職      | 未婚       | 2回目        | 20分      |
| No.2    | 本人 | 20        | 男性       | 20          | 17          | 会社員      | 未婚       | 初回         | 20分      |
| No.3(F) | 父親 | 60        | 男性       | 20          | 7           | 事務職      | 既婚       | 入院中        | 45分      |
| No.3(W) | 妻  | 30        | 男性       | 20          | 7           | 事務職      | 既婚       | 入院中        | 30分      |

F: father, W: wife を示す

内容

- ②病名を聞いたときの思い
- ③これまでに困ったこと
- ④今後に望むこと

4) 分析方法

インタビュー内容は承諾を得て、できるかぎり正確に記録を行った。記録内容をすべて文字化し逐語録を作成した。文章・段落の意味を損なわない程度で区切り、要約し解釈してコード化する。得られたコードを意味内容の類似性に添って、カテゴリー化し、それぞれにネーミングした。

5) 倫理的配慮

岐阜大学大学院医学系研究科倫理審査委員会の承認を受け、書面にて患者、家族の同意を得た。

結果

1. 基本情報

インタビュー対象者は患者本人が2人、患者の家族が2人であった。対象番号 No.3は精神症状のため今回精神科に入院し、情報収集に有益なインタビューは不可能であった。フェール病患者本人の性別は、男性2人、女性1

人であった。インタビュー時間は20~45分であった。詳細は表1で示す。

2. インタビュー結果

インタビュー内容は、「不安」「医療への期待」「社会、医療体制への要望」「病気に対する思い」の4つのカテゴリーがあった。以下カテゴリー別に述べる。文中に使用する記号は、次のとおりである。サブカテゴリーは[]、患者、家族の語りを「」で示し、末尾の番号は表1における対象番号を表す。

1) 不安

【疾患に関する不安】

「症状がなぜ起きているのか、その説明がつけば安心できるけど、説明がつかないのは不安」(No.1)

「睡眠不足だと集中できないし体が疲れたときとか、頭がフワフワしたようなめまいを感じる」(No.1)

「痙攣について理解してほしいと思った。どこでも大きい発作のことを思っている。痙攣にも大きいものと小さいものがあるのに…」(No.1)

「子宮内膜症の手術のときでも、『何かあったらすぐに押してください』と言われたが、大きい発作があったら、ナーソコールも押せないのに」(No.1)

「いろいろな論文があって、症状がい

ずれ進行していくと話もできなくなつて肺炎とか合併して亡くなるみたいなのが書いてあった」(No.2)

「いいことなんて1つもないって…」(No.2)

「いろいろ読んで、自分のなかでは悪いことしか頭に残らなくて…悪いほう悪いほうに考えてしまい、落ち込んだ」(No.2)

「頭が痛いと言われても、どうにもしてやれなかった。自分(父親自身)も頭痛もちなので、そのつらさはわかるつもりだが、頭痛を和らげるため、(本人が)鎮痛剤を20錠も一度に内服して、大丈夫なのか心配しました」(No.3F)

「高校生の頃、女の子に対しても性的虐待的なことがあった」(No.3F)

「頭の中の病気だし、いつどうなるかわからないし、早く死んでしまうかもしれないと不安に思います」(No.3W)

【就業に関する不安】

「これまで仕事に支障があって、何度か転職した」(No.1)

「(患者自身は)働きたい自分と、働けない自分について、責任感が強いぶん、ジレンマに陥っていたのではないかと思っています。(患者にとって)仕事することは家庭を守ることで、仕事がなくなると生き甲斐がなくなるのではないかと思います」(No.3W)

### 【症状の進行に対する介護者の不安】

「精神的なものがくるのは(精神的な症状の出現)想定外のものでした。穏やかな人だったのに、急に怒鳴ったり、顔つきが豹変した。誰彼かまわず、道で女性に声をかけたり、電話番号を聞き回ったりしました」(No.3F)

「家の中でも急に裸になったときは、私よりも(患者の)体型が大きくて、対処できませんでした。まったく動けなければ、どうにかできるけど、動けるから余計大変」(No.3W)

「これからどうなっていくのか(病状の変化)不安。どんどん知能も体力も低下して行くだらうし、ふだんの生活を送るのにも時間に余裕がないとやっていけないだらうし。もともと頑固な性格だから(笑って話す)」(No.3W)

「周り(信仰している教会の人たち)からも『家庭をもったのなら、仕事をし家庭を守らない』と言われ、本人もその言葉にプレッシャーを受けていた気がします。だから病気のことは、本人だけでなく、周りの人みんなが理解してあげなければいけないと思いました」[「豹変して怖い顔になったりするの、本当に本人が気づかないところで起こっているのだから、周りが気づいてあげないといけないと思います」](NO.3W)

## 2) 医療への期待

### 【専門の医師に出会えた安堵感】

「インターネットで調べて、やっと専門の医師にたどり着いた。そのときは、安堵感を感じた。治らない病気だと聞いていても、自分のことをわかってくれる専門の医師に出会えたことがうれしかった。メールでもすぐに返信してもらえたことも安心した」(No.1)

「専門の医師と話すことで、救われた。希望がもてた感じだった」(No.2)

「てんかん発作の予防のためにデパスとか飲んでいるけど、薬のせいで症状が出ているかもしれないと思って、薬の量を自分で調整していた。でも専門の医師から自分で調整しないように言われて止めました」(No.1)

「風邪にも鼻詰まりから重い肺炎まであるように、ファール病だからといって皆同じ進行状態で進むとは限らないと聞いて、納得できた。僕も頭部CTで、石灰化も進行していないようだし、あまり悪く考えなくなりました」(No.2)

### 【専門外の医師に理解してもらいたい思い】

「初めて受診した脳神経外科の先生が、この病気のことをよく理解していなくて、先生のネットワークもなくてつらかった。頭の石灰化には、カルシウムが関与しているだろうから、牛乳を飲むのを控えるように言われ、控えていた。でもこの病気になって血液検査でもカルシウム値が低くなっているわけでもなく、専門の医師からカルシウムを控えて、骨粗鬆症になっても困るから、と言われたので、控えなくてもいいのかと思った。一般の先生にも広くこの病気のことについて理解してほしい」(No.1)

「ファール病がもっと世のなかで認められた病気になれば、自分たちの対応も違っていただのではないかなと思う。息子を追い詰めることもなかった気がします。それは、(本人が)『結婚したのなら、奥さんを養って、赤ちゃんが生まれれば、しっかりしたお父さんにならなければ』と周りから言われていた。本人もその気で頑張りたいのに、職場

では十分な仕事もできないでいた。(本人は)職場に迷惑もかけられないと思って、自分で午前中だけ仕事をして、午後はどこかで時間を過ごして家に帰っていました。それを内緒にしていました。本当は(本人は)決められた仕事に、責任をもって続けたい気持ちがあったと思います。すべてが、本人を追いかけていたのではないかな、と思っています」(No.3F)

## 3) 社会、医療体制への要望

### 【医療体制やサポート体制の整備を希望】

「これまでに転職したけど、今でも職ももっているし、時間内に業務を終了できて無理することもないです。この病気をもって生きていこうと決心したのは、アメリカに1年間留学したときに、私には味方している人がいる、同じ病気の人たちの励みになろうと、自分なりの何かの成果をだそうと思ったからです」(No.1)

「ファール病の研究を進めてほしい」(No.3W)

「ファール病の患者さんや家族と直接、話をして意見交換したい」(No.3W)

「〇〇さん(患者本人)は人の世話をすることが好きだから、障害者施設やデイサービスで、障害者の人たちのなかで過ごしてほしい」(No.3W)

### 【情報入手の困難さ】

「いろいろな病院にかかった」(No.1)

「インターネットで見てもすぐにたどり着かなかった」(No.2)

「専門の医師の存在を知ったのは、自分がインターネットでファール病の講演をされている先生だと知って、(高校生のときだったので)親に受診する手筈を整えてもらった」(No.2)

表2 ● カテゴリー・サブカテゴリーの表

| カテゴリー         | サブカテゴリー                       |
|---------------|-------------------------------|
| 不安            | 疾患に関する不安                      |
|               | 就業に対する不安                      |
|               | 症状の進行に対する介護者の不安               |
| 医療への期待        | 専門の医師に出会えた安堵感                 |
|               | 専門外の医師に理解してもらいたい思い            |
| 社会、医療体制への要望   | 医療体制やサポート体制の整備を希望             |
|               | 情報入手の困難さ                      |
| 病気に対する漠然とした思い | 偶然ファール病と診断されたときの戸惑い           |
|               | 1人だけで闘っている思い                  |
|               | 自分の異常のすべてがファール病に起因しているのではないか？ |

4) 病気に対する漠然とした思い

【偶然ファール病と診断されたときの戸惑い】

「高校生のときに泡を吹いて痙攣を起こして倒れた。そのときに病院を受診し、頭のCTを撮ってもらってわかった」(No.1)

「この病気の診断のきっかけとなったのは、血液のことを考えると異常に気分が悪くなった。会社の新規採用者歓迎の講演で、震災の生々しい話がでて、それを想像するだけで気分が悪くなって、倒れた。その後頭の痛いのが続き、〇県の病院を受診してCTを撮ってもらった。そこで脳が石灰化している治らないファール病と言われた」(No.2)

「幼稚園から小学校の低学年頃に、頭をタオルで縛ってくれと言われた。そのうちに痛みがなくなったのか、自然に寝て2時間くらい寝られたみたいです。そんなことが1週間に1、2回あったように記憶しています。てんかんなのか心配して、初めて病院でCTを撮ってもらい、そこで脳の石灰化を指

摘され専門の病院を受診するよう言われたけど放置していました。高校生のときに交通事故に遭って骨折し、このことも兼ねて、精密検査をしてもらうために、入院したその病院で、初めてファール病と言われました」(No.3F)

【1人だけで闘っている思い】

「病気に関して両親に『私の病気じゃないからわからない』と言われたことを恨んだこともあった。自分じゃなくても私を生んだ責任として、一緒に考えてほしいと思った」(No.1)

【異常のすべてがファール病に起因しているのではないか？】

「子宮内膜症の治療のときに、自分ではわからないほど大声を出していた」「手術のとき、眠たくても寝られなかった。そのときは食事を禁止されていたので、てんかんの薬を飲まなかったからかなあ」

「自分の思っている言葉と、話している言葉が違っているときがあるみたいで心配。電話をしていて前の会社の名前を言って、電話相手が戸惑った。の

で、すぐに電話を切った」(No.1)  
「この病気からくるのでしょうか、よくわからないのですが、高校生のときに受験のこともあり精神的に不安定になって精神科を受診しました。今はそのようなことはなくなりました。ただ血液だけには異常に反応します。それは高校生のときからだった気がします」(No.2)

5) 4つのカテゴリーの関連(表2、図1)

抽出されたカテゴリーは4つで、それぞれのサブカテゴリーは表2のようになった。それぞれのカテゴリーのまとめとカテゴリーの関連は以下のとおりである。

ファール病の患者や家族は、未知の疾患であるファール病がどのように進行し、どんな症状が出現し、将来的に自分がどのようなになるのか、そのことによって就業が続けられるのか、寝たきりになってしまっただけで家族の介護負担が過剰になり迷惑がかかるのではない



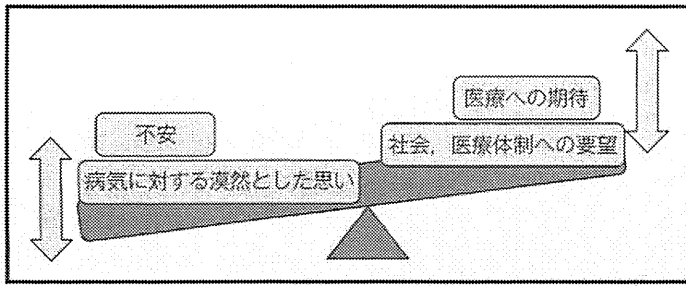


図1 ●カテゴリー概念図

かの不安があった。

そのため患者や家族が専門の医師に出会ったときには、やっと自分の疑問に答えてくれる医師に出会えた安堵感と期待にあふれていた。同時に、専門の医師がいる病院を受診するには、地理的に遠いため、経済、労力、時間の負担が重くのしかかった。それゆえ、専門外の医師にフェール病を理解してもらえればその負担が軽減でき、安心して地域の医療機関を受診できるのではないかと、医療への期待があった。

またフェール病と診断されてから、患者や家族は疾患を理解したくてインターネットなどあらゆる手立てを使って必死に情報の入手を試みた。しかし自身が納得できる情報が得られず、1人で闘っているような孤独感を感じていた。さらに少ない情報のもとで、自分が感じる異常な症状がすべてフェール病に結びついているのではないかと、病気に対する漠然とした思いなどがあり、今後の医療支援体制の確立、整備を希望していた。カテゴリーの関連、心理的狀態を模式化したのが図1である。

## 考察

### 1. 医療体制やサポート体制の整備を希望

#### 1) 体制の整備

[相談支援]や[サポート体制]整備への期待や希望が高いことが理解できた。希少難病であるフェール病と診断されたとき、疾患に関する情報、療養生活の注意点、心のケアなど、難病患者や家族の心のよりどころとなるような相談支援やサポートを受けられる体制が望まれていると考える。

#### 2) 患者会の設立

各県には難病患者相談支援センターが設置されているが、フェール病は特定疾患にも属していないため、団体がない。患者や家族が、「同じ病気の患者さんの家族と話がしたい」という[家族会の必要性]のニーズがあることがわかった。

フェール病と診断をされても、その疾患に関する情報はきわめて少なく、患者や家族は戸惑い、途方に暮れていた。今回のインタビューからも、実際に医師から「牛乳を飲まないように」と指導されたことを考えると、医師です

らフェール病について十分な情報がないことがわかった。そのため患者や家族はさすがの思いで、小さな情報を頼りに確かな情報を求めている。よって同じ病気の患者と話し合う機会を得られる[家族会]は、病気に関する知識の獲得や情報交換が可能となり、他の患者とのつながりを体験をもつことにより孤独感が改善され、同時に不安感や抑うつ感が緩和されるため、設立が必至と考えられる。

以上2点より、できれば行政や医療者が主体となり、ニーズに応じた患者会を立ち上げることが望ましいが、現状すぐには難しい。せめて、情報技術(IT)機器を活用した神経難病の心のケアネットワークなどで患者や家族の声を発信したり、ピアサポートのような会から推進できることが良いのではないかと考える。

## 研究の限界と今後の課題

今回の対象は人数が少なく、必ずしも患者全体像を把握しているわけではない。また疾患概念もあいまいで、診断基準の確立が望まれる。今回の対象患者は典型的、若年患者である。今後、特発性両側性脳内石灰化症(idiopathic bilateral intracranial calcification; IBIC)という切り口から、正確な診断による疾患のカテゴリー別の分類、対象数を増やした検討が必要である。

またその解析においても対象数を増やし、より詳細な質的分析が必要である。現在、主に初診時に行った面談をもとに検討しているが、面談を繰り返す過程での行動の変容など、経時的観察も必要である。しかし、患者は全国から集まり、定期的通院が困難なこと

もあり、今後、研究対象者を電話相談者にも拡大すること、またIT機器を活用した心のケアシステムの構築などが必要と考えられる。

ファール病患者および家族の幅広いニーズをさらに明確にし、患者会の設立も含めて、実質的により良い医療看護の支援体制の確立を目指していきたい。

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<http://www.med.gifu-u.ac.jp/neurology/fahr.html>

## 2012年秋期 リンパ浮腫指導技能者養成講座

■日 程：2012年11月19日(月)～12月8日(土)

\*月曜～金曜：8時～17時50分

\*土曜：8時～12時20分

\*日曜：休講

135時間(座学45時間、実習90時間、実習は修了試験を含む)

■場 所：天神チクモビル(福岡市中央区天神3-10-27)

■受講料：40万円(税別/教材費・受験料を含む)

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るグループもある<sup>11)</sup>。このような異なる結果が出来る理由として考えられることは、給餌しているHFD(含まれる脂肪酸の種類や割合)や飼育環境(≒腸内細菌叢)の違い、また使用しているNKT細胞欠損マウスの違いなどがあげられる。とくに腸内細菌叢は肥満とのかかわりが強く、NKT細胞は腸内細菌叢の形成に関与しているとの報告もある<sup>12)</sup>。なにがこれらの結果の違いをもたらすおま原因となっているのか非常に興味深い。同じく生活習慣病のひとつである動脈硬化症においてはNKT細胞が活性化することでTh1偏倚し、病巣が拡大する<sup>13)</sup>が、これまでの報告は肥満の系で認められるような研究室間の差は少ない。肥満、動脈硬化症のいずれにおいても生体内でどのようなリガンドがNKT細胞を活性化しているのかはいまだ不明である。リガンドの探索を含め、NKT細胞がこれらの生活習慣病のあらたな治療標的となりうるか、さらなる検討が必要であろう。

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#### 神経内科学

### 進むFahr病の病態解明

Remarkable progress of research on Fahr's Disease

#### ≡ Fahr病とは

頭部MRIの普及によって、最近では頭部CTを施行する機会は以前よりは少なくなった。しかし、頭部CTにおいて淡蒼球に石灰化を認めることはけっして珍しいことではない。その頻度、大きさは加齢とともに増大する。これは血管の動脈硬化と関連し、生理的なものと考えられている。一方、このような生理的石灰化とは区別して、原因不明で両側に脳内に石灰化をきたす症例は慣例的に“Fahr病”と呼称されてきた。その症状としては、無症状から頭痛、幼小児期のてんかんや知能発達遅延、思春期における精神症状など多彩であり、平均像をとらえることは難しい。

#### ≡ Fahr病班研究

著者らは平成22(2010)年7月から厚生労働科学研究費補助金難治性疾患克服研究事業のひとつとし

て、Fahr病(特発性両側性大脳基底核・小脳歯状核石灰化症)の病態解明に取り組んできた。全国の神経内科のある病院、小児神経専門医に、一次調査として“原因不明で、頭部CTで両側大脳基底核and/or小脳歯状核に生理的な範囲を超えて病的と判断される石灰化を認める症例”の調査・登録を行った。平成24(2012)年5月末日時点で岐阜大学附属病院へ紹介や直接受診した症例を含め、146名の登録症例がある。しかし、そのなかには副甲状腺機能低下症が判明した症例、通常みられる石灰化とは違った頭部CT画像呈する症例、その後の検索からCockayne症候群、Aicardi-Goutières症候群などがむしろ疑われる症例なども含まれていた。また、初老期の認知症を呈し、脳内石灰化をきたす症例には、剖検所見からびまん性の神経原線維変化が認められる疾患が存在し、小阪によってdif-

fuse neurofibrillary tangles with calcification (DNFC)として報告され<sup>1)</sup>、近年では臨床症例の報告者である柴山とともに、欧米でも小阪・柴山病と呼称されている。Fahr病との関連、鑑別を明確にすることが必要である。

### ≡ あらたな研究展開

Fahr病の疾患概念は不明瞭であり、その使用は適切でないとする意見が多い。海外でも疾患概念として、基底核を軸に Idiopathic Bilateral Ganglionic Calcification (IBGC) という名称が用いられている。そのなかの家族性の症例から遺伝子異常がみつき、IBGC1 (14q13: MGEA6 遺伝子の p. P521A 変異)、IBGC2 (2q37) として報告されてきた。ごく最近、2012年2月12日、『Nature Genetics』に IBGC の家族例7家系(中国3家系、ブラジル3家系、スペイン1家系)と孤発例と思われる4症例に、Ⅲ型ナトリウム依存性リン酸トランスポーター2 (PiT2) をコードする *SLC20A2* の変異がみつき、変異 cDNA を導入したアフリカツメガエルの卵母細胞で、リン酸運搬能の異常が証明された<sup>2)</sup>。著者らの検索においても数例にこの遺伝子に mutation が認められた。病因にリン酸トランスポーターが関与していることは Fahr 病の病態解明へ大きな一歩を踏み出しことになる。

### ≡ 今後の課題と展望

頭部 CT 画像における淡蒼球の石灰化は、病理解剖における小血管における石灰化の程度、臨床症状の重症度とかならずしも相関しない。小血管における石灰化、神経原線維変化を生前にとらえる画像検査法がない。著者らは ICP-MS を用いて髄液中の重金属を測定し、Fahr 病、小阪・柴山病と思われるどちらの症例においてもカルシウム (Ca) ではなく、銅 (Cu)、亜鉛 (Zn)、鉄 (Fe)、マグネシウム (Mg) の増加を認めている<sup>3)</sup>。当初推定されていた小阪・柴山病のリン酸化タウの増加も小阪・柴山病のみならず、Fahr 病、Alzheimer 病で総タウ量の増加が認められ、かならずしも鑑別には役立たないと考えられた。Fahr 病の剖検脳におけるタウの検索は今後の課題のひとつと思われる。また、小阪・柴山病と診断された症例の子供が Fahr 病と思われる臨床症状であったり Fahr 病と小阪・柴山病は同じスペクトラム上にある可能性も考えられる。今後、解決すべき課題は多い。

今回の遺伝子解析から *SLC20A2* の変異がみつき、PiT2 の異常が見出された意義はきわめて大きい。しかし、PiT2 は脳だけに特異的に発現する蛋白質ではない。一方、肺胞微石症ではⅡ型肺胞上皮細胞に発現する type II b sodium phosphate co-transporter の遺伝子 (*SLC34A2*) 変異

が報告されている<sup>4)</sup>。副甲状腺疾患にみられるように組織の Ca 沈着にはリン (P) の値が高いことがまず重要である。リン酸トランスポーターの異常は Ca のみならず、他の重金属の蓄積にも関与することが推測される。家族性 Fahr 病においてはまだ遺伝子異常がみつかっていない家系が多く、今後、石灰化症の原因遺伝子を検索するにはこれまで同定されている 378 種の SLC トランスポーター遺伝子のなかでもターゲットを絞り、リン酸トランスポーター [*SLC20* (type III Na<sup>+</sup>-phosphate cotransporter family: A1, A2), *SLC17A1*, *SLC34* (type II Na<sup>+</sup>-phosphate cotransporter family: A1, A2, A3)] を軸とした検索を重点的に行っていくことも戦略の一つかと思われる。

今後、遺伝子、関連分子を切り口とした分類、病態解明が重要である。そのことはキレート剤やリン酸結合蛋白質などの使用による治療薬の開発につながるものである。

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# Decreased Bioelements Content in the Hair of Patients with Fahr's Disease (Idiopathic Bilateral Calcification in the Brain)

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**Abstract** The remarkable calcification of the basal ganglia and cerebellum has been traditionally called Fahr's disease, but this nomenclature is criticized for including heterogeneous diseases. To determine the pattern of some biological metals in the hair of patients with Fahr's disease, we investigated the levels of 24 bioelements in the hair of 28 patients (17 males and 11 females) with Fahr's disease and compared them with those of three age-, sex-, and living region-matched controls (84 controls in total). Interestingly, we found decreases in the levels of several bioelements [calcium (Ca), copper (Cu), iron (Fe), mercury (Hg), iodine (I), nickel (Ni), phosphate (P), lead (Pb), and selenium (Se)] in the hair of patients. This is in contrast to our previous finding of increases of Cu, Fe, zinc (Zn), and magnesium (Mg) in the cerebrospinal fluid (CSF) of patients.

The decreased level of Cu in the hair was the most prominent and pathognomonic, while the increased level of Cu in the CSF had been found to be the most significant in patients. More significant correlations between two bioelements in the hair were recognized in patients than controls. Although Fahr's disease has been considered to be a heterogenous entity, the significant tendencies of several bioelements in the hair of patients in this study suggest metabolic disorders of bioelements, especially biometals, on the background. Some transporters, especially P transporter such as PiT2, of bioelements will be involved in the different distribution of bioelements in the body of patients.

**Keywords** Fahr's disease · Hair · Calcification · Bioelement · Biometal

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## Introduction

The nomenclature of Fahr's disease has been criticized for including heterogeneous diseases and the disease has presented as a clinically complex syndrome [1]. Recently, the nomenclature of idiopathic bilateral basal ganglia and cerebellar calcification (IBGC) has been used because it shows simply and precisely the condition. Most types of IBGC are sporadic and the etiology still remains unknown. Some familial cases have been reported to be associated with some genetic mutations: 14q (IBGC1), 2q37, and 8p (*SLC20A2*) [2–7].

In Japan, elderly patients showing dementia and calcification of the basal ganglia show diffuse neurofibrillary tangles with calcification and the absence of senile plaques in pathology (called DNTC or Kosaka–Shibayama disease) [8, 9]. A patient with DNTC has recently been reported in the USA [10] and many DNTC patients are assumed to exist in the country. However, it seems difficult to distinguish DNTC from Fahr's

disease and physiological calcification in some elderly patients without autopsy.

In neuropathology, the chemical composition of cerebral stones in a patient with Fahr's disease mainly shows hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)\text{OH}$ ), mucopolysaccharide, and the relatively high levels of trace metals including zinc (Zn), iron (Fe), copper (Cu), magnesium (Mg), lead (Pb), and others [11, 12]. However, there is no apparent explanation for the accumulation of calcium and other metals. We have recently reported high levels of Cu, Zn, Fe, and Mg, but not calcium (Ca), in the cerebrospinal fluid (CSF) of patients with Fahr's disease [13].

Recent great advantages in high-sensitive and reliable trace elements analysis method using inductively coupled plasma mass spectrometry (ICP-MS) have enabled it to be applied for estimating metals and essential minerals in human body, showing that human blood mineral concentration reflect to their levels in hair specimen [14, 15]. As hair sampling is least invasive, we have examined the levels of 24 bioelements in the hair of patients with Fahr's disease and compared them with those of three controls matched in age, sex, and living region to determine if there is a pattern in the levels of some biological metals in the hair of patients with Fahr's disease.

## Material and Methods

### Subjects

We nationally collected hair from patients with idiopathic bilateral calcification of the basal ganglia and/or cerebellum, so-called Fahr's disease, with informed consent. The diagnostic criteria for Fahr's disease in this study were shown in Table 1. The study was approved by the Ethics Committee of the Gifu University Graduate School of Medicine.

Twenty-eight patients (17 males and 11 females) were selected in accordance with our criteria (Table 1). The patients'

mean age $\pm$ standard deviation (SD) was 45.5 $\pm$ 20.3 years. Three sex-, age-, and living region-matched individuals used as control were randomly selected from our database. Therefore, the control group consisted of 51 males and 33 females. The mean $\pm$ SD age was 45.4 $\pm$ 20.0 years.

### Sample Collection and Treatment

Hair was cut at the proximal portion and 75 mg of hair sample was collected from the proximal part and placed in a small paper bag. The hair sample was washed twice with acetone and then with 0.01 % Triton solution, in accordance with the procedure reported by the Hair Analysis Standardization Board. The washed hair sample was mixed with 10 ml of 6.25 % tetramethylammonium hydroxide (TMAH, Tama Chemical, Kawasaki, Japan) and 50  $\mu\text{L}$  of 0.1 % gold solution (SPEX Certi Prep.), and then dissolved at 75 °C with shaking for 2 h. After cooling the solution to room temperature, an internal standard (Sc, Ga, and In) solution was added. After adjusting its volume gravimetrically, the obtained solution was used for mineral analysis [14].

### Determination of Metals in Hair

The mineral levels of 24 bioelements [aluminum (Al), arsenic (As), boron (B), bromine (Br), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), Cu, Fe, germanium (Ge), mercury (Hg), iodine (I), potassium (K), Mg, manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphate (P), Pb, selenium (Se), vanadium (V), and Zn] were measured using ICP-MS (Agilent-7500ce) by the internal standard method and expressed as nanograms per hair. For quality control of the mineral analysis, human hair certified reference materials supplied by the National Institute for Environmental Studies of Japan (NINE CRM) was used in the study [11].

### Statistical Analysis

The levels of 24 bioelements were showed logarithmical normal distributions, so the statistical analyses were done after logarithmic transformation. The levels of all bioelements were compared between patients and controls by using analysis of variance such that matching conditions were considered as the block effect. The correlations among 24 bioelements in respective groups of patients and controls were analyzed by Pearson's correlations coefficients. Moreover, a conditional multiple logistic regression analysis by the forward selection method based on likelihood ratio was employed to determine the influence rate of the bioelements between patients and controls. The significant level of 0.05 was used for all statistical tests (two tailed). Statistical analyses were performed using IBM SPSS Statistics 20.

**Table 1** Diagnostic criteria for Fahr's disease

1. Conspicuous calcification is observed in the basal ganglia and/or dentate nucleus by CT scan.
2. Calcification is bilateral and symmetrical.
3. Idiopathic (unknown cause to the best of our knowledge)
  - (a) To exclude parathyroid diseases, especially hypoparathyroidism: Normal levels of calcium, phosphate and intact parathyroid hormone (iPTH). No Albright's signs: short stature, obese, round faces, short III and IV fingers
  - (b) To exclude metabolic diseases: No pathognomically physiological or developmental disorder
  - (c) To exclude DNTC: No atrophy of the frontal and/or temporal lobe in the CT or MRI scan. No low blood flow in the frontal and/or temporal lobe in the SPECT image. No presenile or senile progressive dementia.

**Table 2** Descriptive statistics for mineral levels in the hair of Fahr's patients and controls

| Element   | Patients (n=28)<br>Geometric mean (range <sup>a</sup> ) | Controls (n=84)<br>Geometric mean (range <sup>a</sup> ) | P value <sup>b</sup> |
|-----------|---|---|----------------------|
| Al (mg/g) | 2.8 (1.1–7.0)   | 3.4 (1.8–6.4)   | 0.174                |
| As (μg/g) | 52.3 (21.1–129.5)                                       | 52.6 (28.6–96.4)  | 0.976                |
| B (μg/g)  | 348.2 (157.0–772.7)                                     | 307.9 (120.4–787.8)                                     | 0.458                |
| Br (mg/g) | 2.7 (1.0–6.9)   | 3.3 (1.1–9.7)   | 0.334                |
| Ca (mg/g) | 266.4 (133.0–533.5)                                     | 384.9 (196.5–753.9)                                     | 0.008                |
| Cd (μg/g) | 5.0 (1.2–20.7)  | 5.7 (2.0–16.3)  | 0.592                |
| Co (μg/g) | 2.3 (0.8–6.5)   | 3.5 (1.3–9.5)   | 0.069                |
| Cr (μg/g) | 50.9 (30.5–84.7)  | 39.3 (17.2–89.9)  | 0.075                |
| Cu (mg/g) | 13.2 (7.7–22.7)   | 21.3 (11.7–38.6)  | <0.001               |
| Fe (mg/g) | 4.3 (3.4–5.5)   | 5.3 (3.9–7.2)   | 0.002                |
| Ge (μg/g) | 47.0 (24.4–90.3)  | 49.1 (19.1–126.0)                                       | 0.798                |
| Hg (mg/g) | 1.9 (0.6–6.0)   | 3.5 (1.9–6.6)   | <0.001               |
| I (μg/g)  | 181.7 (75.8–435.5)                                      | 317.0 (108.6–924.9)                                     | 0.012                |
| K (mg/g)  | 14.4 (3.3–62.7)   | 14.9 (5.1–43.2)   | 0.886                |
| Mg (mg/g) | 30.6 (16.2–58.1)  | 39.3 (20.7–74.6)  | 0.065                |
| Mn (μg/g) | 83.7 (34.6–202.4)                                       | 90.5 (45.0–182.2)                                       | 0.623                |
| Mo (μg/g) | 27.3 (10.7–69.8)  | 32.2 (20.5–50.8)  | 0.206                |
| Na (mg/g) | 21.6 (5.3–88.0)   | 18.5 (6.6–51.6)   | 0.537                |
| Ni (μg/g) | 64.3 (21.9–189.0)                                       | 158.8 (59.1–426.4)                                      | <0.001               |
| P (mg/g)  | 117.0 (91.8–149.2)                                      | 137.4 (110.1–171.6)                                     | 0.002                |
| Pb (μg/g) | 194.4 (42.6–887.4)                                      | 345.6 (141.5–843.8)                                     | 0.014                |
| Se (μg/g) | 557.4 (439.5–707.0)                                     | 650.4 (533.8–792.5)                                     | 0.001                |
| V (μg/g)  | 7.7 (3.5–16.6)  | 7.8 (2.9–21.1)  | 0.926                |
| Zn (mg/g) | 131.9 (110.4–157.7)                                     | 137.6 (115.7–163.7)                                     | 0.268                |

<sup>a</sup>Geometric mean±geometric standard deviation range

<sup>b</sup>The analysis of variance with patients/control effects and block effects for matching was used for group comparisons

## Result

The descriptive statistics for bioelements levels in the hair of patients and controls are shown in Table 2. The geometric mean of the level of Ca was the highest value (patients,

266.4 mg/g; controls, 384.9 mg/g) and those of Zn and P were the second and third highest values, respectively. The mineral levels of Ca, Cu, Fe, Hg, Ni, P, and Se in the hair of the patients were significantly decreased than those of controls ( $p<0.01$ ). The mineral levels of I and Pb in the patient's hair were also decreased than those of controls ( $p<0.05$ ).

**Table 3** Correlations among the metals in each of Fahr's patients and controls

|           | Patients (n=28) |         | Controls (n=84) |         |
|-----------|-----------------|---------|-----------------|---------|
|           | r               | P value | r               | P value |
| Al vs. Mn | 0.63            | <0.001  | 0.30            | 0.005   |
| Al vs. V  | 0.62            | <0.001  | 0.51            | <0.001  |
| As vs. Br | 0.61            | <0.001  | -0.13           | 0.244   |
| Ca vs. Mg | 0.82            | <0.001  | 0.80            | <0.001  |
| Cd vs. Mn | 0.61            | <0.001  | 0.28            | 0.009   |
| Cd vs. Pb | 0.62            | <0.001  | 0.35            | <0.001  |
| Co vs. Mn | 0.63            | <0.001  | 0.40            | <0.001  |
| Fe vs. Mn | 0.63            | <0.001  | 0.60            | <0.001  |
| K vs. Na  | 0.83            | <0.001  | 0.76            | <0.001  |

The analyses used the log-transformed data  
r Pearson's correlation coefficient

The correlations among 24 biominerals were investigated by Pearson's correlation coefficients, and we picked up only the correlation coefficients having absolute values more than 0.6. Table 3 shows the correlation coefficients in each of patients and controls. All significant correlations were

**Table 4** Adjusted odds ratio (OR) and 95 % confidence interval (CI) of Fahr's disease from conditional multivariate logistic regression

| Element | OR                   | 95 % CI                      | P value | Wald |
|---------|----------------------|------------------------------|---------|------|
| Cu      | $9.0 \times 10^{-5}$ | $2.1 \times 10^{-7}$ –0.039  | 0.003   | 9.01 |
| Ni      | 0.022                | 0.001–0.438                  | 0.012   | 6.24 |
| Hg      | 0.028                | 0.002–0.467                  | 0.013   | 6.18 |
| Se      | $4.8 \times 10^{-8}$ | $3.4 \times 10^{-14}$ –0.067 | 0.020   | 5.45 |

OR odds ratio, 95 % CI 95 % confidence interval

The analysis used the log-transformed data such that the units of the original data were μg/g

positively correlated. The correlations between Ca and Mg, Fe and Mn, and K and Na were significant and more than 0.6 in both patient and control groups. Moreover, correlations between Al and Mn, Al and V, Cd and Mn, Cd and Pb, and Co and Mn were significant in patients group. In particular, the correlation between As and Br in patients group showed significance ( $r=0.61$ ,  $p<0.001$ ), while that of control group showed insignificance ( $r=-0.13$ ,  $p=0.244$ ).

Table 4 shows the result of conditional multivariate logistic regression analysis by the forward selection method based on likelihood ratio. The decreased levels of Cu, Hg, Ni, and Se were significant in association with Fahr's disease and the level of Cu was the most significant ( $OR\ 9.0 \times 10^{-5}$ ,  $p=0.003$ ).

## Discussion and Conclusion

High levels of the trace metals Cu, Fe, Mg, Pb, and Zn have been reported in cerebral stones, which mainly contain Ca in patients with Fahr's disease [11, 12]. We have found high levels of Cu, Fe, Mg, and Zn in the CSF of patients with Fahr's disease for the first time [13]. The change in CSF might reflect the mineral accumulation of cerebral stones in the brain. However, there is as yet no explanation for the normal level of Ca in the CSF of the patients with Fahr's disease. The serum levels of Ca, Cu, Mg, and Zn were within normal ranges and there were no reports on abnormal Ca metabolism nor abnormal levels of Cu, Zn, Fe, Mg, and other minerals in the sera of patients with Fahr's disease to the best of our knowledge.

In this study, unexpectedly, the levels of Ca, Cu, Fe, Hg, I, Ni, P, Pb, and Se in the hair of the patients with Fahr's disease were lower than those in the controls. This is in contrast to the increase in the levels of Cu, Fe, Mg, and Zn in CSF in patients with Fahr's disease [13]. This may reflect the different bioelements distributions in the human body due to some abnormality of a transporter that might be associated with P and other metals including Ca. We could not explain the imbalance of bioelements in the human body at the present time.

Studies of metals in the body have been reported in patients with Alzheimer's disease [16], Parkinson disease [17], and autism [18]. However, no correlation between the metal levels in the sera and in the hair or an explanation for the imbalance of metals among the parts of the body has been given yet. The imbalance remains to be elucidated in these neurodegenerative diseases.

Recently, mutations of *SLC20A2*, encoding type III sodium-dependent phosphate transporter 2 (PiT2), have been found in familial IBGC, also known as Fahr's disease, in China. PiT2 is important in P transport activity [7] and P is the most important element in calcification. Hypoparathyroidism shows conspicuous calcification in the brain, which

is very similar to the calcification observed in Fahr's disease. The high P level is supposed to be important in the accumulation of Ca and other minerals.

In addition, mutations of *SLC30A10* encoding ZnT-10, a Zn transporter, had recently been observed in patients with Parkinsonism, hypermanganesemia, hepatic cirrhosis, and polycytopenia in an autosomal recessive inheritance [19]. Mn intoxication is well-known to result in Parkinsonism. This study shows that SLCA10, also known as ZnT-10, also plays a role as a Mn transporter in the human body.

Interestingly, more significant correlations between bioelements have been recognized in patients with Fahr's disease than controls. This may reflect different patterns of bioelements due to interrupted transporters of bioelements. Representatively, while the level of Cu in CSF was most significantly increased in patients with Fahr's disease, the decreased level of Cu in the hair was the most prominent and pathognomonic. The clarification of the mutations, functions, and tissue-specific distributions of some bioelements, especially P, transporters in the future will explain the decrease in the levels of some bioelements in the hair of patients with Fahr's disease.

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# Roles and Therapeutic Potential of Metallothioneins in Neurodegenerative Diseases

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**Abstract:** Metallothionein (MT) is a small molecular and multi-functional protein containing four atoms of copper (Cu) and three atoms of zinc (Zn) per molecule. It was isolated from the horse kidney in 1957 and half a century has passed since then. Although MT was found to work as a modulator of Zn and induce anti-oxidant reaction, the precise functions and its functional mechanisms remain to be elucidated. Over the years, a new isoform of MT, MT-III (also called growth inhibitory factor (GIF)), has been found in the brain, which was markedly diminished in the brain of Alzheimer's disease (AD). Many new findings on MT have been discovered in neurodegenerative diseases other than AD such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), prion disease, brain trauma, brain ischemia, and psychiatric diseases. In ALS in particular, MTs were markedly diminished in the spinal cord of patients with ALS. Initially, MT, which easily binds to cadmium (Cd) and copper (Cu), was considered to be toxic to our bodies. Molecular biological technologies enabled the production of recombinant MT saturated with zinc (Zn). MT has a high potential for the treatment of neurodegenerative diseases such as ALS, AD, and PD owing to its various functions including anti-oxidant properties and modulators not only for Zn but for Cu in the extra- and intracellular spaces. On the other hand, there are still various problems on MT to be elucidated in detail, including their binding proteins and functional mechanisms.

**Keywords:** Metallothionein, amyotrophic lateral sclerosis, Alzheimer's Disease, Parkinson's Disease, Fahr's Disease.

## INTRODUCTION

Metallothionein (MT) is a small molecule and is considered to have multiple functions such as maintaining zinc (Zn) and copper (Cu) homeostasis, detoxifying cadmium (Cd) and mercury (Hg), regulating the biosynthesis and activity of Zn-binding proteins such as Zn-dependent transcription factors, protecting against reactive oxygen species (ROS), and minimizing the side effects of chemotherapeutic drugs [1]. Although MT had been considered to be a medicine, the strong affinity with toxic heavy metals including Cd and Cu had been thought to have detrimental effects on bodies. Recombinant MT proteins could be produced using various molecular biological techniques. They are saturated with Zn and free of Cd and Cu (Zn<sub>7</sub>-MT-III). Mammalian MTs are thought to be composed of four isoforms (MT-I to IV). MT-I and MT-II are found in all tissues of the body. Similarities in nucleotide and amino acid sequences make it difficult to distinguish them indisputably by cDNA probes and antibodies (therefore they are abbreviated as MT-I/II). MT-III possesses additionally seven amino acids and exists predominantly in the central nervous system (CNS). MT-III was first characterized as an inhibitory substance for unknown neurotrophic factors in Alzheimer's disease (AD) [2]. MT-IV is found exclusively in stratified squamous epithelia [3]. The ratio of MT isoforms in the brain remains to be elucidated.

One study showed that the ratio of MT mRNA expression levels in the mouse brain is 100: 50: 70 for MT-I : MT-II : MT-III, respectively [4]. Some researchers suspect that MT-III is much more abundant in the brain (personal communication), as MT-III is mainly expressed in CNS [5] and strongly protect against ROS, particularly hydroxyl radicals [6]. MT is considered to be associated with the pathogenesis and progression of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), AD, Parkinson's disease (PD) [1], and Fahr's disease (FD) [7]. Taken together, MTs are promising therapeutic candidates for neurodegenerative diseases [1]. Here, we discuss the roles of MT and some metals, and the therapeutic potential of MT in ALS, PD, AD, and FD.

## AMYOTROPHIC LATERAL SCLEROSIS (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease that is characterized by selective cortical and spinal motor neuron degeneration. The majority of ALS cases are sporadic ALS (SALS), and approximately 10% of ALS cases are familial ALS (FALS). Genes known to cause ALS are *superoxide dismutase 1 (SOD1)* (15%-20%) [8], *ANG* encoding angiogenin [9], *TARDBP* encoding the TAR DNA-binding protein 43 (TDP-43) (~5%) [10], the fused in sarcoma/translated in liposarcoma gene (*FUS*, also known as *TLS*) [11, 12], and, recently, *OPTN* encoding optineurin [13]. The hypotheses underlying ALS pathogenesis regarding the mechanisms are oxidative damage, axonal strangulation from neurofilamentous disorganization, toxicity from intracellular aggregates and/or fail-

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ure of protein folding or degradation, and repetitive motor neuron firing and subsequent excitotoxic death due to the mishandling of glutamate [1, 14]. Other mechanisms have been reported including heavy-metal toxicity, neurotrophic factor dysfunction, and endoplasmic reticulum stress [15, 16]. Despite these studies, the etiology of SALS remains unclear.

Crossing G93A SOD1 Tg mice with MT-III knockout mice accelerated the progression of ALS in their progenies [17, 18]. The expression levels of MT-III mRNA were decreased in the spinal cords of patients with SALS [19]. We previously showed that the expression of MTs was diminished in the spinal cords of patients with SALS, which suggested that they are heavily involved in the pathogenesis of SALS [20]. Recent studies have suggested that physical exercise has a beneficial effect on disease progression in ALS patients [21] and G93A SOD1 mutant transgenic (G93A SOD1 Tg) mice, a FALS mouse model [22]. Physical exercise increases the levels of MT-I, MT-II, and MT-III in the spinal cords of normal mice [23]. The gene therapy with an adenovirus vector encoding the MT-III gene prevented neurodegeneration following facial nerve avulsion and stab wounds [24, 25]. Our preliminary report shows the neuroprotective effect and the prolongation of survival time in G93A SOD1 Tg mice by MT-III administration at the time of disease onset by the retrograde gene delivery from skeletal muscles to the motor neurons of the spinal cord [26].

Kanias and Kapaki reported that the levels of Cu and Zn in CSF were higher in patients with ALS (age > 40) than in older controls (age > 40) as determined by atomic absorption spectrophotometry [27]. Studies on the spinal cord of G93A SOD-1 transgenic mice revealed high levels of Cu and labile Zn [28, 29]. Ammonium tetrathiomolybdate, a Cu-chelating drug, has been found to delay onset, prolong survival, and slow disease progression in G93A SOD-1 Tg mice [30]. Cu and Zn are considered to play pivotal roles in the development of ALS.

Several molecules and several important mechanisms on the onset and progression of ALS have been found in recent studies. The most important for the treatment of ALS is to find the kingpin molecule in the mechanism of ALS. MTs seem to locate near it. We proposed a mechanism of ALS associated with MT in 2004 [1] and the principle still works.

### ALZHEIMER'S DISEASE (AD)

AD is characterized by the accumulation of  $\beta$ -amyloid (A $\beta$ ) plaques, neurofibrillary tangles, and neuronal death in the neocortex. Although the molecular mechanisms underlying AD pathogenesis remain to be elucidated, the formation of A $\beta$  accumulation of  $\beta$ -amyloid (A $\beta$ ) plaques, neurofibrill assumed to lead to neuronal death in the neocortex.

The down-regulation of MT-III has been found in human AD brains [2]. The most important point concerning MT-III is not simply the decrease in the level of MT-III throughout the brain, but the marked decrease of MT-III in that of in reactive astrocytes around A $\beta$  plaques. This marked decrease in reactive astrocytes may lead to (1) the outgrowth of neurites and ultimately dystrophic neurites as proposed first by Uchida et al, (2) an increase in the level of free Zn<sup>2+</sup> which

would promote the accumulation of the A $\beta$  protein in senile plaques, (3) an increase in the level of free Cu<sup>+</sup> which would promote the Fenton reaction, and (4) an increase in the level of free hydroxyl radicals which cause cell damage. These processes would ultimately result in neuronal death. Neuronal loss also results in the decrease in the level of MT-III in the whole brain. Therefore, it is important to distinguish the decrease in the level of MT-III in reactive astrocytes, which may be considered as an accelerating factor for the disease, from the decrease in the level of MT-III caused by neuronal death. We proposed a mechanism of AD associated with MT in 1998 and the principal still works [1].

A recent study has showed that serum copper level is associated with MMSE score worsening in patients with AD [29]. Zn level was also reported to be increased in the human AD-affected cortex [31]. A study on Japanese American men suggested that Zn and Cu modulate A $\beta$ -42 levels in CSF [32]. Therefore, both Cu and Zn are considered to be key metals in the progression of AD.

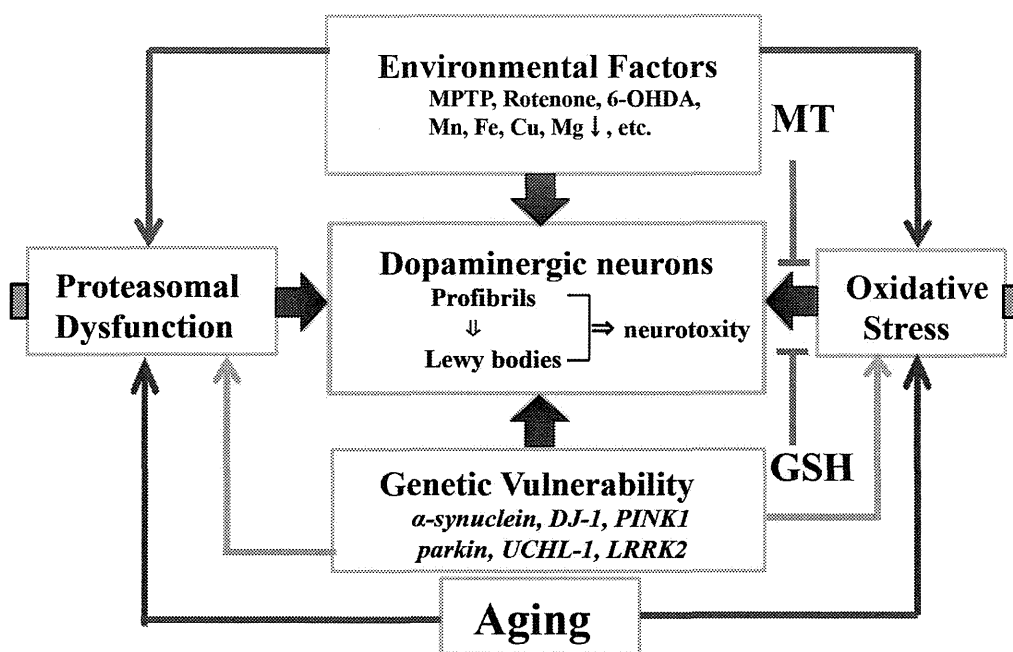
Taken together, MT-III is still considered to be the kingpin molecule in the mechanism of AD, associated with the modulation of Cu and Zn.

### PARKINSON'S DISEASE (PD)

PD is characterized by the degradation of dopaminergic cells in the presence of Lewy bodies (LBs). LBs have a high concentration of  $\alpha$ -synuclein. Dopaminergic cells die owing to a combination of the following factors: 1) genetic vulnerability, (2) oxidative stress, (3) proteosomal dysfunction, (4), and environmental factors. The mutations of  *$\alpha$ -synuclein* [33], *parkin* [34], *UCHL1* [35], *DJ-1* [36], *PINK1* [37] and *LRKK2* [38] affect the biochemical processing of  $\alpha$ -synuclein and cause PD [39]. Although the cause of PD remains unclear, oxidative stress and proteosomal processing during aging play important roles in sporadic PD. (Fig. 1)

The effects of MT-I/II on the dopaminergic neurotoxicity of 6-hydroxydopamine (6-OHDA) were examined in MT-I/IIKO mice by intraventricular injection of 6-OHDA. The loss of dopaminergic neurons in the substantianigra induced by the administration of 6-OHDA was significantly aggravated in the MT-I/II KO mice. This indicates that MT-I/II exerts neuroprotective effects against dopaminergic neurotoxicity of 6-OHDA at the nigral cell body by scavenging free radicals [40].

The expression level of MT-III mRNA was examined in the basal ganglia of hemi-parkinsonian rats lesioned by 6-OHDA in order to clarify the changes in MT-III expression and the regulation by levodopa in dopaminergic neurodegeneration. In normal rats, levodopa/carbidopa significantly increased the expression level of striatal MT-III mRNA in a dose-dependent manner 24 hr after their administration. The induction of MT-III mRNA expression peaked 24 hr after levodopa/carbidopa administration. The levodopa-induced MT-III mRNA expression might represent a complementary reaction against oxidative stress. In experiments using hemi-parkinsonian rats, the expression level of MT-III mRNA was significantly decreased on the 6-OHDA-lesioned side in the striatum 24 hr after the treatment. On the 6-OHDA-lesioned side, levodopa/carbidopa treatment did not increase in the



**Fig. (1). Schematic Representation of Proposed Mechanism of PD Associated with MT.** Genetic factors, environmental factors, and aging contribute to the loss of cellular function and death. The proteasomal dysfunction and oxidative stress are considered to promote neurotoxicity through the formation of profibrils and Lewy bodies in dopaminergic neurons. Anti-oxidative agents (MT = metallothionein, GSH = glutathione) prevent oxidative stress in dopaminergic neurons.

expression level of MT-III mRNA. These findings indicate that free radical scavenging potency including MT-III is reduced in the parkinsonian brain, and that levodopa fails to induce MT-III mRNA expression to consequently accelerate the progression of PD [41].

Mn intoxication has been well known to cause Parkinsonism. A survey suggested that chronic occupational exposure to metals, particularly Mn or Cu, is associated with PD [42]. Low-level Mg intake over generations was shown to cause the degeneration of the substantia nigra in rats [43]. MT is supposed to prevent the progression of PD by protecting against oxidative stress.

**FAHR’S DISEASE (FD)**

Patients with marked calcification of the basal ganglia and cerebellum have been traditionally referred to as showing ‘Fahr’s disease’, but the nomenclature has been criticized for including heterogeneous etiologies [44]. We have described the cases of three patients with ‘Fahr’s disease’ (idiopathic bilateral striato-pallido-dentate calcinosis (IBSPDC)). We found significantly increased levels of copper (Cu), zinc (Zn), iron (Fe), and magnesium (Mg) by inductively coupled plasma mass spectrometry in the CSF of these three patients [4]. The elderly patients with dementia and calcification of the basal ganglia in Japan were reported to show diffuse neurofibrillary tangles and the absence of senile plaques in the autopsy [45, 46]. There is no specific and effective treatment for IBSPDC at present. The markedly increased levels of Cu and Zn (4 and 3 times higher than the levels in controls, respectively) in CSF suggest a possibility of treatment using MT, which possesses 4 atoms of Cu and 3

atoms of Zn per molecule and is a modulator for both Cu and Zn.

**CONCLUSION REMARKS**

MT was first isolated from the horse kidney by Margoshes and Vallee in 1957 [47] and half a century has passed since then. Over the years, an isoform of MT-III was found in AD by Uchida *et al.* in 1991 [2]. However, there are still many enigma on MT including its precise function, its mechanism, and binding proteins. MT works as a modulator of biological reactions in the human body. First, MT is a modulator not only Zn but also Cu in the intra and extracellular space [48]. Second, MT forms a protective barrier against oxidative stress [49]. Third, MT works as a modulator of NFκB [50].

MT-III, in particular, possesses unique properties other than the common features of MTs, including the inhibition of new neurite outgrowth of neurons *in vitro* [1]. A discrepancy between the abundance of MT-III protein in astrocytes and that of MT-III mRNA in neuron in the brain remains to be elucidated [51-53]. MT-III does not exist exclusively in the central nervous system. MT-III also exists in the reproductive and urinary stems including the testis and prostate, tongue [5, 54], and the epithelial cells of the blood vessels throughout the body. MT-III is also called growth inhibitory factor [2]. Thus, it appears that MT-III is a negative factor for growth and regeneration, where as MT-I/II appears to be a positive for growth and regeneration. However, both MT-I/II and M-III have protective effects on neurons [26, 55, 56]. MT-III showed the double-edged effects such as some tissue-protective and adverse effects according to the dosage