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 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

This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (H23-Nanbyo-Ippan-106) (to I.H.).

Disclosure statement

No potential conflicts of interest are disclosed.

References

1 Delacour A. Ossification des capillaires du cerveau. *Ann Med Psychol (Paris)* 1850; **2**: 458–461.
2 Yulmaz K, Cem C, Nevzat K, Cagatay O. Intracranial calcification on CT. *Diagn Interv Radiol* 2010; **16**: 263–269.
3 Manyam BV. What is and what is not 'Fahr's disease'. *Parkinsonism Relat Disord* 2005; **11**: 73–80.
4 Asai H, Kameyama M. Basal ganglia calcification. A review. *Neurol Med* 1982; **16**: 585–594.
5 Okudera H, Hara H, Kobayashi S, Okada K, Shimizu K. Evaluation of the intracranial calcification associated with aging on computerized tomography. *No To Shinkei* 1986; **38**: 129–133.
6 Eskandary H, Sabba M, Khajehpour F, Eskandari M. Incidental findings in brain computed tomography scans of 3000 head trauma patients. *Surg Neurol* 2005; **63**: 550–553.
7 Brannan TS, Burger AA, Chaudhary MY. Bilateral basal ganglia calcification visualized on CT scan. *J Neurol Neurosurg Psychiatry* 1980; **43**: 403–406.
8 Harrington MG, Macpherson P, McIntosh WB, Allam BF, Bone I. The significance of the incidental findings of basal ganglia calcification on computed tomography. *J Neurol Neurosurg Psychiatry* 1981; **44**: 1168–1170.
9 Kosaka K. Diffuse neurofibrillary tangles with calcification: a new presenile dementia. *J Neurol Neurosurg Psychiatry* 1994; **57**: 594–596.
10 Shibayama H, Kobayashi H, Nakagawa M *et al.* Non-Alzheimer non-Pick dementia with Fahr's syndrome. *Clin Neuropathol* 1992; **11**: 237–250.
11 Nanda S, Bhatt SP, Pamula J, Woodruff WW, Fowler M, Miller D. Diffuse neurofibrillary tangles with calcification (DNTC): Kosaka-Shibayama disease in America. *Am J Alzheimers Dis Other Demen* 2007; **22**: 535–537.
12 Kwak R, Takeuchi F, Ito S, Kadoya S. Intracranial psydiological calcification on computed tomography (part 1): calcification of pineal region. *No to Shinkei* 1998; **40**: 569–574.
13 Duckett S, Galle P, Escourolle R, Poirier J, Hauw JJ. Presence of zinc, alminum, magnesium in striopalledodentate (SPD) calcification (Fahr's disease): electron probe study. *Acta Neuropathol* 1977; **38**: 7–10.
14 Kobayashi S, Yamadori I, Miki H, Ohmori M. Idiopathic nonarteriosclerotic cerebral calcification (Fahr's disease): an electron microscopic study. *Acta Neuropathol* 1987; **73**: 62–66.

投稿受領No. 910

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

堀田みゆき*1　　保住　功*2

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，無症状の状態で発見されることが多い[1)2)]。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，治療法もない」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。

*1 岐阜大学医学部附属病院医療連携センター
*2 岐阜薬科大学薬物治療学研究室
Hotta Miyuki, Hozumi Isao

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

表1●患者・家族の属性

対象番号	属性	年齢（歳台）	患者性別	患者年齢（歳台）	発症年齢（歳）	患者職業	患者結婚	外来受診回数	面談時間
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。ファ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]

「症状がなぜ起こっているのか，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，ナース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って肺炎とか合併して亡くなるみたいなことが書いてあった」(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ろうし，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ろうし。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，仕事をして家庭を守らないと』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，本人だけでなくて，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は，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のではないかと思う。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，本人を追い詰めていたのではないか，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)

「○○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，○県の病院に受診して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に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て家族の介護負担が過剰になり迷惑がかかるのではない

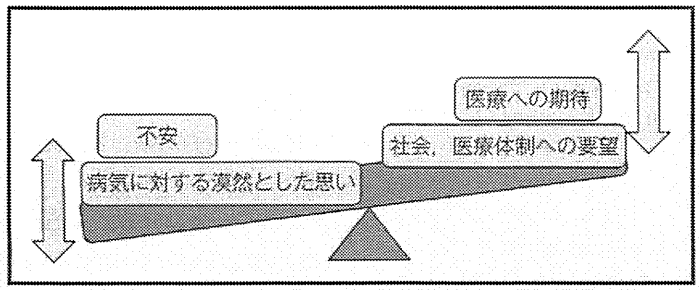

図１◎カテゴリー概念図

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応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，専門外の医師にファ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に受診できるのではないかという，医療への期待があった。

またファ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ァ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，心理的状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。希少難病であるファ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れ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，ファ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。

ファ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，医師でさらファ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すがる思いで，小さな情報を頼りに確かな情報を求めていた。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（idipapthic 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。

本研究は，厚生労働科学研究費補助金　難治性疾患克服研究事業　ファール病(特発性両側性大脳基底核・小脳歯状核石灰化症)研究班の助成を受けて行われた。

●引用文献●

1) Manyam, B.V.：What is and what is not 'Fahr's disease'. Parkinsonism Relat Disord, 11(2)：73-80, 2005.
2) Hozumi, I., Kohmura, A., Kimura, A., et al.：High Levels of Copper, Zinc, Iron and Magnesium, but not Calcium, in the Cerebrospinal Fluid of Patients with Fahr's Disease. Case Rep Neurol, 2(2)：46-51, 2010.

参考

岐阜大学大学院医学系研究科神経内科・老年学分野：ファール病・関連脳内石灰化症のため，受診をお考えの皆様へ
http://www.med.gifu-u.ac.jp/neurology/fahr.html

るグループもある[11]．このような異なる結果が出来す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[12]．なにがこれらの結果の違いをもたらすおもな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[13]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．

1) 滋賀医科大学附属病院TOPICS，**12**：2001年8月1日．
2) Weisberg, S. P. et al.: Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.*, **112**：1796-1808, 2003.
3) Nishimura, S. et al.: CD8+ T effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.*, **15**：914-920, 2009.
4) Van Kaer, L.: NKT cells: T lymphocytes with innate effector functions. *Curr. Opin. Immunol.*, **19**：3768-3781, 2007.
5) Caspar-Bauguil, S. et al.: Adipose tissue as an ancestral immune organ：site-specific change in obesity. *FEBS Lett.*, **579**(17)：3487-3492, 2005.
6) Lynch, L. et al.: Invariant NKT cells and CD1d(+)cells amass in human omentum and are depleted in patients with cancer and obesity. *Eur. J. Immunol.*, **39**(7)：1893-1901, 2009.
7) Satoh, M. et al.: Type Ⅱ NKT cells stimulate diet-induced obesity by mediating adipose tissue inflammation, steatohepatitis and insulin resistance. *PLoS One*, **7**(2)：e30568, 2012.
8) Jahng, A. et al.: Prevention of autoimmunity by targeting a distinct, non-invariant CD1d-reactive T cell population reactive to sulfatide. *J. Exp. Med.*, **199**：947-957, 2004.
9) Wu, L. et al.: Activation of invariant natural killer T cells by lipid excess promotes tissue inflammation, insulin resistance, and hepatic steatosis in obese mice. *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas. 1200498109：2012.
10) Ji, Y. et al.: Activation of natural killer T cells promotes M2 macrophage polarization in adipose tissue and improves systemic glucose tolerance via the IL-4/STAT6 signaling axis in obesity. *J. Biol. Chem.*, **287**：13561-13571, 2012.
11) Kotas, M. E. et al.: Impact of CD1d deficiency on metabolism. *PLoS One*, **6**(9)：e25478, 2011.
12) Edward, E. S. et al.: Cd1d-dependent regulation of bacterial colonization in the intestine of mice. *J. Clin. Invest.*, **119**(5)：1241-1250, 2009.
13) Nakai, Y. et al.: Natural killer T cells accelerate atherogenesis in mice. *Blood*, **104**(7)：2051-2059, 2004.

佐藤　雅，岩渕和也／
Masashi SATOH and Kazuya IWABUCHI
北里大学医学部免疫学

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，原因不明で両側性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(DNTC)として報告され[1]，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かり，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*の変異がみつかり，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[2]．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[3]．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*の変異がみつかり，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typeⅡb sodium phosphate co-transporterの遺伝子(*SLC34A2*)変異が報告されている[4]．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* (typeⅢ Na^+-phosphate cotransporter family：*A1*, *A2*), *SLC17A1*, *SLC34*(typeⅡ Na^+-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．

1) Kosaka, K.: *JNNP*, **57**：594-596, 1994.
2) Wang, C. et al.: *Nat. Genet.*, **44**：254-256, 2012.
3) Hozumi, I. et al.: *Case Rep. Neurol.*, **2**：46-51, 2010.
4) Hugun et al.: *Am. J. Respir. Crit. Care*, **175**：263-268, 2007.

保住　功／Isao HOZUMI
岐阜薬科大学薬物治療学

*　　*　　*

Biol Trace Elem Res (2013) 151:9–13
DOI 10.1007/s12011-012-9529-z

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

Mari Takagi · Kazuhiro Ozawa · Hiroshi Yasuda · Mitsuko Douke · Kazunori Hashimoto · Yuichi Hayashi · Takashi Inuzuka · Isao Hozumi

Received: 8 September 2012 / Accepted: 12 October 2012 / Published online: 24 October 2012

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

M. Takagi · M. Douke · K. Hashimoto · Y. Hayashi · I. Hozumi (✉)
Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University,
1-25-4 Daigaku-Nishi,
Gifu 501-1196, Japan
e-mail: hozumi@gifu-pu.ac.jp

K. Ozawa
Nursing Collaboration Center, Gifu College of Nursing,
3047-1 Egirachou,
Hashima 501-6295, Japan

H. Yasuda
Research Laboratory, La Belle Vie Inc.,
8-4 Nihonbashi-Tomizawachou,
Chuo-ku, Tokyo 103-0006, Japan

K. Hashimoto · Y. Hayashi · T. Inuzuka · I. Hozumi
Department of Neurology and Geriatrics, Gifu University, Graduate School of Medicine,
1-1 Yanagido,
Gifu 501-1194, Japan

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 (Ca5(PO4)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±standard deviation (SD) was 45.5±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±SD age was 45.4±20.0 years.

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 pathognomonically 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.

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

Element	Patients (n=28) Geometric mean (range[a])	Controls (n=84) Geometric mean (range[a])	P value[b]
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

[a]Geometric mean±geometric standard deviation range

[b]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).

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 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

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	9.0×10^{-5}	2.1×10^{-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	4.8×10^{-8}	3.4×10^{-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 9.0×10^{-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.

Acknowledgments We thank Dr. T. Imai of Sapporo Medical University Hospital, Dr. H. Ishida of Izumi Municipal Hospital, Dr. K. Iyoda of Hiroshima City Hospital, Dr. N. Kamisato of Akita Rosai Hospital, Dr. J. Kawata of Shonankamakura General Hospital, Dr. S. Kobayashi of Sapporo Medical University Hospital, Dr. A. Kume of Kume Clinic, Dr. K. Maeda of Shiga Hospital, Dr. Y. Manabe of Okayama Medical Center, Dr. M. Matsuo of Saga University Hospital, Dr. E. Miyashiro of Kaseitamura Hospital, Dr. K. Miyazaki of Shiseikai Daini Hospital, Dr. M. Mori of Jichi Medical University Hospital, Dr. T. Motoshima of Chiba Children's Hospital, Dr. H. Murai of Iizuka Hospital, Dr. K. Shioya of Miyazaki Higashi Hospital, Dr. K. Nagashima of Public Nanokaichi Hospital, Dr. T. Nishigaki of Osaka Police Hospital, Dr. H. Nishimura of Nishinomiya Kyoritu Neurosurgical Hospital, Dr. H. Nishiura of Isahaya Health Insurance General Hospital, Dr. H. Shimada of Osaka City University Hospital, Dr. N. Suzuki of Tohoku University Hospital, Dr. Y. Taguchi of Toyama University Hospital, Dr. S. Takeshita of Yokohama City University Medical Center, Dr. E. Tsuda of Sapporo Medical University Hospital, Dr. S. Tsuneishi of Medical Welfare Center Kizuna, Dr. T. Uemichi of Kinki Central Hospital, and Dr. M. Yamada of Omori Red Cross Hospital for providing the patient specimens. We also thank Professor H. Hara of Gifu Pharmaceutical University, and Professor M. Sato of Aichi Gakuin University, for their helpful suggestions. This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (H23-Nanbyo-Ippan-106 (to IH).

References

1. Manyam BV (2005) What is and what is not 'Fahr's disease'. Parkinson and Related Disord 11:73–80
2. Geschwind DH, Loginov M, Stern JM (1999) Identification of a locus on chromosome 14q for idiopathic basal ganglia calcification (Fahr disease). Am J Hum Genet 65:764–772
3. Lemos RR, Oliveira DF, Zatz M et al (2011) Population and computational analysis of the MGEA6 P521A variation as a risk

factor for familial idiopathic basal ganglia calcification (Fahr's disease). J Mol Neurosci 43:333–336
4. Oliveira JR, Spiteri E, Sobrido MJ et al (2004) Genetic heterogeneity in familial idiopathic basal ganglia calcification (Fahr disease). Neurology 63:2165–2167
5. Volpato CB, De Grandi A, Buffone E et al (2009) 2q37 as a susceptibility locus for idiopathic basal ganglia calcification (IBGC) in a large South Tyrolean family. J Mol Neurosci 39:346–353
6. Dai X, Gao Y, Xu Z et al (2010) Identification of a novel genetic locus on chromosome 8p21.1-q11.23 for idiopathic basal ganglia calcification. Am J Med Genet B Neuropsychiatr Genet 7:1305–1310
7. Wang C, Li Y, Shi L, Ren J et al (2012) Mutations in *SLC20A2* link familial idiopathic basal ganglia calcification with phosphate homeostasis. Nat Genet 44:254–256
8. Kosaka K (1994) Diffuse neurofibrillary tangles with calcification: a new presenile dementia. J Neurol Neurosurg Psychiatry 57:594–596
9. Shibayama H, Kobayashi H, Nakagawa M et al (1992) Non-Alzheimer non-Pick dementia with Fahr's syndrome. Clin Neuropathol 11:237–250
10. Nanda S, Bhatt SP, Pamula J et al (2007) Diffuse neurofibrillary tangles with calcification (DNTC): Kosaka–Shibayama disease in America. Am J Alzheimer's Disease and Other Dementias 22:535–537
11. Smeyers-Verbeke J, Michotte Y, Pelsmaeckers J et al (1975) The chemical composition of idiopathic nonarteriosclerotic cerebral calcifications. Neurology 25:48–57
12. Duckett S, Galle P, Escourolle R et al (1977) Presence of zinc, aluminum, magnesium in striopalledodentate (SPD) calcifications (Fahr's disease): electron probe study. Acta Neuropathol 38:7–10
13. Hozumi I, Kohmura A, Kimura A et al (2010) High levels of copper, zinc, iron and magnesium, but not calcium, in the cerebrospinal fluid of patients with Fahr's disease. Case Rep Neurol 2:46–51
14. Yasuda H, Yoshida K, Yasuda Y, Tsutsui T (2011) Infantile zinc deficiency: association with autism spectrum disorders. Scientific Reports 1, Article number:129
15. Ochi A, Ishimura E, Tsujimoto Y, Kakiya R, Tabata T, Mori K, Shoji T, Yasuda H, Nishizawa Y, Inaba M (2011) Trace elements in the hair of hemodialysis patients. Biol Trace Elem Res 143:825–834
16. Shore D, Henkin RI, Nelson NR et al (1984) Hair and serum copper, zinc, calcium, and magnesium concentrations in Alzheimer-type dementia. Am Geriatr Soc 32:892–895
17. Forte G, Alimonti A, Violante N et al (2005) Calcium, copper iron, magnesium, silicon and zinc content of hair in Parkinson's disease. J Trace Elem Med Biol 19:195–201
18. Yasuda H, Yonashiro T, Yoshida K et al (2005) A mineral imbalance in children with autistic disorders. Biomed Res Trace Elements 16:285–292
19. Tuschl K, Clayton PT, Gospe SM et al (2012) Syndrome of hepatic cirrhosis, dystonia, polycythemia, and hypermanganesemia caused by mutations in SLC30A10, a manganese transporter in man. Am J Hum Genet 90:1–10

Send Orders of Reprints at reprints@benthamscience.net

Current Pharmaceutical Biotechnology, 2013, *14*, 000-000

Roles and Therapeutic Potential of Metallothioneinsin Neurodegenerative Diseases

Isao Hozumi*

Department of Neurology and Geriatrics, Gifu University, Graduate School of Medicine, Yanagido, Gifu 501-1194, Japan

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.

INTRODUTION

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_7-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 MTmRNA 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-

*Address correspondence to the author at the Department of Neurology and Geriatrics, Gifu University, Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan; Tel: +81 58 230 6253; Fax: +81 58 230 6256; E-mail: ihozumi@gifu-u.ac.jp

1389-2010/13 $58.00+.00

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-1Tg 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 β-amyloid (Aβ) plaques, neurofibrillary tangles, and neuronal death in the neocortex. Although the molecular mechanisms underlying AD pathogenesis remain to be elucidated, the formation of Aatee accumulation of β-amyloid (Aβ) 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 Aβ plaques. This marked decrease in reactive astrocytes may lead to (1) the outgrowth of neurites and ultimately dystrophic neuritesas proposed first by Uchida et al, (2) an increase in the level of free Zn^{2+} which would promote the accumulation of the Aβ protein in senile plaques, (3) an increase in the level of free Cu^{+} 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 Aβ-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 α-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 *α-synuclein* [33], *parkin* [34], *UCHL1* [35], *DJ-1* [36], *PINK1* [37] and *LRRK2* [38] affect the biochemical processing of α-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 neurotoxity 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 andthe 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 hemiparkinsonian 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

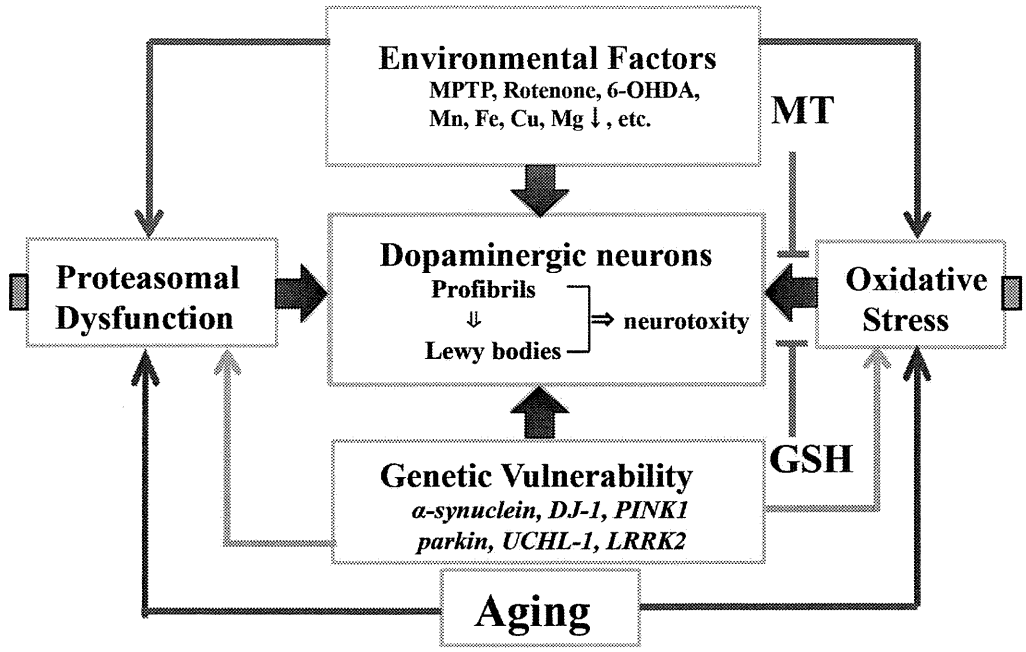

Fig. (1). Schematic Representation of Proposed Mechanism of PD Associated with MT. Genetic factors, environmental factors, and aging contribute to the loss of cellular function and death. The proteasomal dysfunction and oxidative stress are considered to promote neurotoxity through the formation of profibrils and Lewy bodies in dopaminergic neurons. Anti-oxidative agents (MT = metallotionein, 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 substantial 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.* in1991 [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

for stab wound injury and its regeneration [57]. 'Why do MT isoforms exist?' There is no answer yet; however, MT-I/II is thought to be an acutely reactive (anti-inflammatory) protein, while the reaction of MT-III is slower than that of MT-I/II and MT-III continues to work longer, based on the observation of the stab wounds in the rat brain [58].

Similarly there are two types of fiber (type I and type II) in the muscle. The type I muscle fibers react slower and more continuous than the type II muscle fibers. The ratio of type I muscle fibers to type II fibers differs among muscles according to the function. The existence of two types of fibers in the muscle is similar to that of isoforms of MT.

MT-III is abundant in the CNS. It is sure that MT-III plays important roles in the brain and in the progression of neurodegenerative diseases such as ALS, AD, PD, FD, as well asprion disease [59], brain trauma [58], brain ischemia [60], and psychiatric disorders [61]. A combination of MT-I/II and MT-III will be good tools for the treatment of neurodegenerative diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by the grants-in-aids from the Community for Communication of Technology of Gifu University, the Ministry of Education, Culture, Sports, Science and Technology of Japan (Basic Research (B) 19390151), and the Ministry of Health, Labour and Welfare of Japan (Control and Prevention of Intractable Diseases, H22-Intractable-General-202), Japan. The author is grateful for the continuous support and encouraging advice from all members of Tokai Metallothionein Research Group: Professor T. Inuzuka and all his colleagues in Department of Neurology and Geriatrics, Gifu University, Graduate School of Medicine, Gifu, Japan; Professor H. Hara and his colleagues in Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, Gifu, Japan; Professor H. Nagase of Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University, Gifu, Japan; Chief Investigator Y. Uchida of Gene Expression Research Group, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; and Professor M. Sato and his colleagues in Laboratory of Pharmaceutical Health Sciences, School of Pharmacy, Aichi Gakuin University, Nagoya, Japan.

ABBREVIATIONS

AD	=	Alzheimer's disease
ALS	=	Amyotrophic lateral sclerosis
Cd	=	Cadmium
Cu	=	Copper
FD	=	Fahr's disease
GSH	=	Glutathione
Hg	=	Mercury
MT	=	Metallothionein
PD	=	Parkinson's disease
ROS	=	Reactive oxygen species
Zn	=	Zinc

REFERENCES

[1] Hozumi, I.; Asanuma, M.; Yamada, M.; Uchida, Y. Metallothioneins and neurodegenerative diseases. *J. Health Sci.*, **2004**, *50*(4), 323-331.

[2] Uchida, Y.; Takio, K.; Titani, K.; Ihara, Y.; Tomonaga, M. The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein. *Neuron*, **1991**, *7*(2), 337-347.

[3] Quaife, C.J.; Findley, S.D.; Erickson, J.C.; Froelick, J.G.; Kelly, E.J.; Zambrowics, B.P.; Palmiter, R.D. Induction of a new metallothionein isoform (MT-IV) occurs during differentiation of stratified squamous epithelia. *Biochemistry*, **1994**, *33*(23), 7250-7259.

[4] Choudhuri, S.; Kramer, K. K.; Berman, N. E. J.; Dalton, T. P.; Andrews, G. K.; Klaassen, C. D. Constitutive expression of metallothioneingenes in mouse brain. *Toxicol. Appl. Pharmacol.*, **1995**, *131*(1), 144-154.

[5] Hozumi, I.; Suzuki, J.S.; Kanazawa, H.; Hara, A.; Saio, M.; Inuzuka, T.; Miyairi, S.; Naganuma, A.; Tohyama, C. Metallothionein-3 is expressed in the brain and various peripheral organs of the rat. *Neurosci. Lett.*, **2008**, *438*(1), 54-58.

[6] Uchida, Y.; Gomi. F.; Masumizu, T.; Miura, Y. Growth inhibitory factor prevents neurite extension and the death of cortical neurons caused by high oxygen exposure through hydroxyl radical scavenging. *J. Biol. Chem.*, **2002**, *277*(35), 32353-32259.

[7] Hozumi, I.; Koumura, A.; Kimura, A.; Hasegawa, T.; Honda, A.; Hayashi, Y.; Hashimoto, K.; Yamada, M.; Sakurai, T.; Tanaka, Y., Satoh, M.; Inuzuka T. High levels of copper, zinc, iron, and magnesium, but not calcium in the cerebrospinal fluid of patients with Fahr's disease. *Case Rept. Neurol.*, **2010**, *2*, 46-51.

[8] Rosen, D.R.; Siddique, T.; Patterson, D.; Figlewicz, D. A.; Sapp, P.; Hentati, A.; Donaldson, D.; Goto, J.; O'Regan, J. P.; Deng, H. X.; Rahmani, Z.; Krizus, A.; McKenna-Yasek, D.; Cayabyab, A.; Gaston, S. M.; Berger, R.; Tanzi, R. E.; Halperin, J. J.; Herzfeldt, B.; Bergh, R. V D.; Hung, W. Y.; Bird, T.; Deng, G.; Mulder, D. W.; Smyth, C.; Laing, N. G.; Soriano, E.; Pericak–Vance, M. A.; Haines, J.; Rouleau, G. A.; Gusella, J. S.; Horvitz, H. R.; Brown R. H. Jr. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature*, **1993**, *362*, 59-62.

[9] Greenway, M.J.; Andersen, P. M.; Russ, C.; Ennis, S.;Cashman, S.;Donaghy, C.; Patterson, V.;Swingler, R.; Kieran, D.;Prehn, J.; Morrison, K. E.; Green, A.;Acharya, K. R.; Brown, R. H. Jr.; Hardiman, O. ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. *Nat. Genet.*, **2006**, *38*(4), 411-413.

[10] Sreedharan, J.; Blair, I. P.;Tripathi, V. B.; Hu, X.; Vance, C.; Rogelj, B.; Ackerley, S.; Durnall, J.C.; Williams, K. L.;Buratti, E.;Baralle, F.; de Belleroche, J.; Mitchell, J. D.; Leigh, P. N.; Al-Chalabi, A.; Miller, C. C.; Nicholson, G.; Shaw, C. E. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science*, **2008**, *319*(5870),1668-1672.

[11] Kwiatkowski, T. J. Jr.; Bosco, D. A.; Leclerc, A. L.; Tamrazian, E.; Vanderburg, C. R.; Russ, C.; Davis, A.; Gilchrist, J.; Kasarskis, E. J.; Munsat, T.; Valdmanis, P.; Rouleau, G. A.; Hosler, B. A.; Cortelli, P.; de Jong, P. J.; Yoshinaga, Y.; Haines, J. L.; Pericak-Vance, M. A.; Yan, J.; Ticozzi, N.;Siddique, T.; McKenna-Yasek, D.; Sapp, P. C.; Horvitz, H. R., Landers, J. E.; Brown, R. H. Jr. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science*, 2009, *323*, 1205-1208.

[12] Vance, C.; Rogelj, B.; Hortobagyi, T.; De Vos, K. J.; Nishimura, A. L.; Sreedharan, J.; Hu, X.; Smith, B.; Ruddy, D.; Wright, P.; Ganesalingam, J.; Williams, K. L.; Tripathi, V.; Al-Saraj, S., Al-Chalabi, A.; Leigh, P. N.; Blair, I. P.; Nicholson, G.; de Belleroche, J.; Gallo, J. M.; Miller, C. C.; Shaw, C. E. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science*, **2009**, *323*(5918),1208-1211.

[13] Maruyama, H.; Morino, H.; Ito, H.; Izumi, Y.; Kato, H.; Watanabe. Y.; Kinoshita, Y.; Kamada, M.; Nodera, H.; Suzuki, H.; Komure, O.; Matsuura, S.; Kobatake, K.; Morimoto, N.; Abe, K.; Suzuki, N.; Aoki, M.; Kawata, A.; Hirai, T.; Kato, T.; Ogasawara, K.; Hi-

rano, A.; Takumi, T.; Kusaka, H.; Hagiwara,, K.; Kaji, R.; Kawakami, H. Mutation of optineurin in amyotrophic lateral sclerosis. *Nature*, **2010**, *465*(7295), 223-226.

[14] Cleveland, D. W.; Rothstein, J. D. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat. Rev. Neurosci.*, **2001**, *2*, 806-819.

[15] Boillée, S.; Velde, C.V.; Cleveland, D. W. ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron*, **2006**, *52*(1), 39-59.

[16] Ito, Y.; Yamada, M.; Tanaka, H.; Aida, K.; Tsuruma, K.; Shimazawa, M.; Hozumi, I.; Inuzuka, T.; Takahashi, H.; Hara, H. Involvement of CHOP, an ER-stress apoptptic mediator, in both human sporadic ALS and ALS model mice. *Neurobiol. Dis.*, **2009**, *36*(3) 470-476.

[17] Nagano, S.; Satoh, M.; Sumi, H.; Fujimura, H.;Tohyama, C.; Yanagihara, T.; Sakoda, S. Reduction of metallothioneins promotes the disease expression of familial amyotrophic lateral sclerosis mice in a dose-dependent manner. *Eur. J. Neurosci.,* **2001**, *13*(7), 1363-1370.

[18] Puttaparthi, K.; Gitomer, W. L.; Krishnan, U.; Son, M.; Rajendran, B.; Elliott, J. L. Disease progression in a transgenic model of familial amyotrophic lateral sclerosis is dependent on both neuronal and non-neuronal zinc binding proteins. *J. Neurosci.,* **2002**, *22*(20), 8790-8796.

[19] Ishigaki, S.; Niwa, J.; Ando, Y.; Yoshihara. T.; Sawada, K.; Doyu, M.; Yamamoto, M.; Kato, K.; Yotsumoto, Y.; Sobue, G. Differentially expressed genes in sporadic amyotrophic lateral sclerosis spinal cords--screening by molecular indexing and subsequent cDNA microarray analysis. *FEBS Lett.*, **2002**, *531*(2), 354-358.

[20] Hozumi, I.; Yamada, M.; Uchida, Y.; Ozawa, K.; Takahashi, H.; Inuzuka, T. The expression ofmetallothioneins is diminished in the spinal cords of patients with sporadic ALS. *Amyotroph. Lateral. Scler.*, **2008b**, *9*, 294-298.

[21] McCrate, M. E.; Kaspar, B. K. Physical activity and neuroprotection in amyotrophic lateral sclerosis. *Neuromolecular Med.*, **2008**, *10*(2), 108-117.

[22] Kirkinezos, I. G.; Hernandez, D.; Bradley, W. G.; Moraes, C. T. Regular exercise is beneficial to a mouse model of amyotrophic lateral sclerosis. *Ann. Neurol.*, **2003**, *53*(6), 804-807.

[23] Hashimoto, K.; Hayashi, Y.; Inuzuka, T.; Hozumi, I. Exercise induces metallothioneins in mouse spinal cord. *Neuroscience,* **2009**, *163*(1), 244-251.

[24] Sakamoto, T.; Kawazoe, Y.; Uchida, Y.;Hozumi, I.; Inuzuka, T.; Watabe, K. Growth inhibitory factor prevents degeneration of injured adult rat motoneurons. *Neuroreport,* **2003**, *14*(17), 2147-2151.

[25] Hozumi, I.; Uchida, Y.; Watabe, K.; Sakamoto, T.; Inuzuka, T. Growth inhibitory factor (GIF) can protect from brain damage due to stab wounds in rat brain. *Neurosci. Lett.,* **2006**, *395*(3), 220-223.

[26] Hashimoto, K.; Hayashi, Y.;Inuzuka, T.; Hozumi, I. () Growth inhibitory factor inhibits the progression of amyotrophic lateral sclerosis model mice. *Neurology*, **2010**, *74*(Suppl 2), A436

[27] Kanias, D.; Kapaki, E. Trace elements, age, and sex in amyotrophic lateral sclerosis disease. () *Biol. Trace Element Res.*, **1997**, *56*(2), 187-201.

[28] Tokuda, E.; Ono, S., Ishige, K.; Naganuma, A.; Ito, Y.; Suzuki, T. () Metallothionein proteins expression, copper and zinc concentrations, and lipid peroxidation level in a rodent model for amyotrophic lateral sclerosis. *Toxicology*, **2007**, *229*, 33-41.

[29] Kim, J.; Kim, T. Y.; Hwang, J. J.; Lee, Y. Y.; Shin, J. H.;Gwag, B. J.; Koh, J. Y. () Accumulation of labile zinc in neurons and astrocytes in the spinal cords of G93A SOD-1 transgenic mice. *Neurobiol. Dis.*, **2009**, *34*, 221-229.

[30] Tokuda, E.; Ono, S.; Ishige, K.; Naganuma, A.; Ito, Y.; Suzuki, T. () Ammonium tetrathiomolybdate delays onset, prolongs survivals, and slows progression of disease in a mouse model for amyotrophic lateral sclerosis. *Exper. Neurol.,* **2008**, *34*, 122-128.

[31] Squitti, R.; Bressi, F.; Pasqualetti, P.; Bonomini, C.; Ghidoni, R.; Binetti, G.; Gassetta, F.; Moffa, F.; Ventriglia, M.; Vernieri, F.; Rossini, P. M. () Longitudinal prognostic value of serum "free" copper in patients with Alzheimer disease. *Neurology*, **2009**, *72*(1), 50-55.

[32] Religa, D.; Strozyk, D.; Cherny, R. A.; Volitakis, I.; Haroutunian, V.; Winblad, B.; Naslund, J.; Bush, A. I. () Elevated cortical zinc in Alzheimer disease. *Neurology*, 2006, *67*(1), 69-75.

[33] Zarranz, J. J.; Alegre, J.; Gómez-Esteban, J. C.; Lezcano, E.;Ros, R.; Ampuero, I.; Vidal, L.; Hoenicka, J.; Rodriguez, O.; Atarés, B.; Llorens, V.; Tortosa, E. G.;de Ser, T.; Muñoz, D. J. () The new mutation, E46K, of α-synuclein causes parkinson and Lewy body dementia. *Ann. Neurol.*, **2003**, *55*(2), 164-173.

[34] Kitada, T.; Asakawa, S.; Hattori, N.; Matsumine, H.; Yamamura, Y.; Minoshima, S.; Yokochi, M.; Mizuno, Y.; Shimizu, N. () Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, **1998**, *392*, 605–608.

[35] Maraganore, D. M.; Lesnick, T. G.; Elbaz, A.; Chartier-Harlin, M. C.; Gasser, T.; Krüger, R.; Hattori, N.; Mellick, G. D.; Quattrone, A.; Satoh J.; Toda, T.; Wang, J.; Ioannidis, J. P.; de Andrade, M.; Rocca, W. A.; UCHL1 Global Genetics Consortium. () UCHL1 is a Parkinson's disease susceptibility gene. *Ann. Neurol.*, **2004**, *55*(4): 512-21.

[36] Canet-Avilés, R. M.; Wilson, M. A.; Miller, D. W.; Ahmad. R.; McLendon, C.; Bandyopadhyay, S.; Baptista, M. J.; Ringe, D.; Petsko, G. A.; Cookson, M. R. () The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc. Natl. Acad. Sci. U S A.,* **2004**, *101*(24), 9103-9108.

[37] Valent, E. M.; Sergio Salvi, S.; Ialongo, T,; Marongiu, R,;Elia, A. E.; Caputo, V.; Romito, L.; Albanese, A.; Dallapiccola, B.;Bentivoglio, A. R. ()PINK1 mutations are associated with sporadic early-onset parkinsonism. *Ann. Neurol.*, **2004**, *56*(3),336-341.

[38] Smith, W. W.; Pei, Z.; Jiang, H.; Moore, D. J.; Liang, Y.; West, A. B.; Dawson, V. L.; Dawson, T. M.; Ross, C. A. (2005) Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc. Natl. Acad. Sci. U S A*, *102*(51),18676-18681.

[39] DeLong, M. R.; Juncos, J. L. Parkinson's disease and other movement disorders. In Harrison's principals of internal medicine,16th. edition; McGraw-Hill, Ed.; Kasper, D.L.; Braunwald, E.; Fauci, A.S.; Hauser, S. L.; Longo, D. L.; Jameson, J. L.: New York, **2005**; pp. 2406-2418.

[40] Asanuma, M.; Miyazaki, I.; Higashi, Y.; Tanaka, K.; Haque, M. E.; Fujita, N.; Ogawa, N. () Aggravation of 6-hydroxydopamine-induced dopaminergic lesions in metallothionein-I and -II knockout mouse brain. *Neurosci.Lett.*, **2002**, *327*, 61-67.

[41] Miyazaki, I.; Sogawab, A. C.; Asanumaa, M.; Higashi, Y.; Tanaka K.; Nakanishi, T.; Ogawa, N. () Expression of metallothionein-III mRNA and its regulation by levodopa in the basal ganglia of hemiparkinsonian rats. *Neurosci. Lett.*, **2000**, *293*, 65-68.

[42] Gorell, J. M.; Johnson, C. C.;Rybicki, B. A.; Peterson, E. L.;Kortsha, G. X.; Brown, G. G.; Richardson, R. J. () Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. *Neurotoxicol.,* **1999**, *20*(2-3), 239-247.

[43] Oyanagi, K.; Kawakami, E.; Kikuchi-Horie, K.;Ohara, K.; Ogata, K.;Takahama, S.; Wada, M.;Kihira, T.; Yasui, M. () Magnesium deficiency over generations in rats with special references to the pathogenesis of the parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. *Neuropathol.,* **2006**, *26*(2), 115-128.

[44] Manyam, B. V. () What is and what is not 'Fahr's disease'. *Parkinson relat. disord.*, **2005**, *11*(2),73-80.

[45] Shibayama, H.; Kobayashi., H.; Nakagawa, M.; Yamada, K.; Iwata, H.; Iwai, K.; Takeuchi, T.; Mu-Qune, X.; Ishihara, R.; Iwase, S.; Kitoh, J. () Non-Alzheimer non-Pick dementia with Fahr's syndrome. *Clinical Neuropathol.*, 1992, *11*(5), 237-50.

[46] Kosaka, K. () Diffuse neurofibrillary tangles with calcification: a new presenile dementia. *J. Neurol. Neurosurg. Psychiatr.*, **1994**, *57*(5), 594-6.

[47] Margoshes, M.; Vallee, B. L. () A cadmium protein from equine kidney cortex. *J. Am. Chem. Soc.*, **1957**, *79*(19), 4813-4814.

[48] Miyayama, T.; Suzuki, K. T.; Ogra, Y. () Copper accumulation and compartmentalization in mouse fibroblast lacking metallothionein and copper chaperone. *Atox1. Toxicol. Appl. Pharm.*, **2009**, *237*, 205-213.

[49] Sato, M.; and Bremner, I. () Oxygen free radicals and metallothionein. *Free Rad. Biol. Med.*, **1993**, *14* (3), 325-337.

[50] Kim, C. H.; Kim, K. J.; Lee, J and Ahn, Y. S. () Zinc-induced NF-K. J.; Lee, can be modulated by changes in the intracellular metallothionein level. *Toxicol. Appl. Pharm.*, **2003**, *190*, 186-196.

[51] Hozumi, I.; Inuzuka, T.; Ishiguro, H.; Hiraiwa, M.; Uchida, Y.; Tsuji, S. () Immunoreactivity of growth inhibitory factor in normal rat brain and after stab wounds –an immunocytochemical study using confocal laser scan microscope. *Brain Res.*, **1996**, *741*, 197-204.

[52] Anezaki, T.; Ishiguro, H.; Hozumi, I.; Inuzuka, T.; Hiraiwa, M.; Kobayashi, H.; Yuguchi, T.; Wanaka, A.; Uda, Y.; Miyatake, T.;

Tohyama, M. () Expression of growth inhibitory factor (GIF) in normal and injured rat brains. *Neurochem. Int.*, **1995**, *27*, 89-94.

[53] Yamada, M.; Hayashi, S.; Hozumi, I.; Tsuji, S.; Takahashi, H. () Subcellular localization of growth inhibitory factor in rat brain: light and electron microscopic immunohistochemical studies. *Brain Res.*, **1996**, *735*(2), 257-264.

[54] Moffatt, P.; Seguin, C. () Expression of the gene encoding metallothionein-3 inorgans of the reproductive system. *DNA Cell Biol.,* **1998**, *17*, 501–510.

[55] Montoliu, C, Monfort, P.; Carrasco, J.; Palacios, O.; Capdevila, M.; Hidalgo, J.; Felipo, V. () Metallothionein-III prevents glutamate and nitric oxide neutotoxicity in primary cultures of cerebellar neurons. *J. Neurochem.*, **2000**, *75*, 266-273.

[56] Uchida, Y.; Gomi, F.; Masumizu, T.; Miura Y. () Growth inhibitory factor prevents neurite extension and the death of cortical neurons caused by high oxygen exposure through hydroxyl radical scavenging. *J. Biol. Chem.,* **2002**, *277*, 32353-32359.

[57] Hozumi, I.; Uchida, Y.;Watabe, K.; Sakamoto, T.; Inuzuka, T. () Growth inhibitory factor (GIF) can protect from brain damage due to stab wounds in rat brain. *Neurosci.Lett.*, **2006**, *395*: 220-223.

[58] Hozumi, I.; Inuzuka, T., H.; Hiraiwa, M.; Uchida, Y.; Anezaki, T.; Ishiguro, H.; Kobayashi H.; Uda, Y., Miyatake, T.; Tsuji, S. () Changes of growth inhibitory factor after stab wounds in rat brain. *Brain Res.*, **1995**, *688*, 143-248.

[59] Kawashima, T.; Doh-uraa, K.: Torisu, M.; Uchida, Y.;Furuta, A.; Iwaki, T. () Differential expression of metallothioneins in human prion diseases. Dement. *Geriatr.Cogn.Disord.*, **2000**, *11* (5), 251-262.

[60] Koumura, A.; Hamanaka, J.; Shimazawa, M.; , Honda, M.; Tsurumaa, K.; Uchida, Y.; Hozumi, I.; Satoh, M.; Inuzuka, T.; Hara, H. () Metallothionein-III knockout mice aggravates the neuronal damage after transient focal cerebral ischemia. *Brain Res.*, **2009**, *1292*(25), 148-154.

[61] Koumura, A., Kakefuda, K.; Honda, A.; Ito, Y.; Tsuruma, K.; Shimazawa, M.; Uchida, Y., Hozumi, I.; Satoh, M.; Inuzuka, T.; Hara, H. () Metallothionein-3 deficient mice exhibit abnormalities of psychiatric behaviors. *Neurosci.Lett.*, **2009**, *467*(1), 11-14.

Received: ???????????? **Revised:** ???????????? **Accepted:** ????????????