あると示されたこと，また記憶障害などの有害作用を減らすために低電流のECT装置の開発も行われたこと，などの理由から，1990年代からは優れた治療法として再評価されている．そのため，記憶障害などの有害作用への不信感は根強いものの[*55]，ECTは，精神医学の標準的な治療法の一つとなっており，米国精神医学会のマニュアル[*56]によれば，緊張病状態，重度の大うつ病，双極性障害などには積極的に用いられるべきとされる．

ただし，ECTは，全身けいれんというてんかん発作によって脳にある種のショック状態を引き起こすことで治療効果を得るという着想を元にした「ショック療法」であり，インスリン注射によって低血糖ショックを引き起こすインスリン昏睡療法[*57]（M・ザーケル〈M. Sakel〉，1933年）やペンチレンテトラゾール（商品名はカルジアゾール，メトラゾール）によって全身けいれんを起こさせる療法[*58]（L・メデュナ〈L. Meduna〉，1935年）の改良版ともいえる．その点では，電気刺激という手法を使ってはいるが，脳内の特定の部位ないし特定の神経回路での情報処理に電気刺激によって影響を与えるという意味での生理学的介入とは異なったものとみるべきかもしれない．

一方，脳内での特定の情報処理を生理学的にコントロールすることで精神疾患を治療する手法も最近いくつか開発されている．だが，その多くは実験段階であり，効果も有害作用も余りわかっていない．したがって，新規治療法の研究開発の倫理として以外に，特別な倫理学的問題を提起するかどうかは定かではない．

パーキンソン病の治療に用いられているDBSは，物理的介入の節で紹介したとおり，うつ状態などの有害作用を引き起こす場合があることから，精神疾患に対する臨床応用が行われつつある．内包前脚刺激による強迫性障害（Obsessive-Compulsive Disorder：OCD）の治療[*59]や前帯状皮質（25野）刺激による難治性うつ病の治療[*60]が試みられている．

1988年に，難治性てんかんの治療法の一つとして開発された迷走神経刺激法（Vagal Nerve Stimulation：VNS）[*61]は，DBSと同様のペースメーカのような機器を体内に植え込んで頸部にある自律神経の一種である迷走神経を刺激する手法である[*62]．抗てんかん薬はしばしば気分変調ないし安定化の作用を持ち，双極性障害や大うつ病の治療に用いられることがある．そのため，VNSについても難治性大うつ病に対する使用が試みられた[*63]．2005年には，米国で難治性大うつ病に対する治療として認可されたものの，その有効性には疑問も呈されている[*64]．

1985年に開発された経頭蓋的磁気刺激法（TMS）[*65]は，頭部に磁気コイルを置いてパルス電流を流すことで磁場を発生させ，脳内に誘導電流を生み出すものである．DBSやVNSのような外科的処置を全く必要としないで脳に生理学的介入を行う点に特徴がある．治療目的の場合は，パルス電流を反復する手法（反復TMS（repetitive TMS：rTMS））が用いられることが多い．脳に電流を流すという点ではECTと似ているため[*66]，1993年から難治性の大うつ病に対する治療が試みられた[*67]．米国では2008年に難治性大うつ病に対する治療として認可されている．きわめて有効という報告[*68]が当初にはあったものの，大規模な追試で同様の結果は再現できていない[*69]．

そのほかに，頭部に陽極と陰極の電極を当てて，数ミリアンペア以内の微弱電流を流すことで脳に影響を与える手法（経頭蓋的直流刺激法〈Transcranial Direct Current Stimulation：tDCS〉）[*70]も，1960年代からうつ状態の治療法として試みられている[*71]．

結語——物理学的・化学的・生理学的な介入に共通するもの

最初に設定した問いに戻ろう．本章でたどってきた神経科学的な脳への介入のさまざまな手法は「先端的」だろうか．否，少なくとも21世紀初頭という現時点においては，そうではない．なぜなら，物理学的・化学的・生理学的な介入手法に共通している考え方，つまり脳への介入によって行動変容を起こすという発想そのものは，人間を精神と身体に二分割（心身二元論）し，身体は一種の機械（人間機械論）であると見なした上で，身体の一部である脳という臓器を精神の座として取り扱う近代医学の構図[*72]とぴったりと一致しているからだ．その意味では，脳への介入による行動変容は，現代社会の支配的な価値観と医療観に変更を迫るようなものではない．

こうした脳と行動の関連についてのこの考え方をもっとも明確に主張したことで知られるのは，19世紀プロイセンの精神医学者W・グリージンガー（Wilhelm Griesinger）である[*73]．

> 精神異常という現象は，どの器官に属するものなのか？　したがって，精神異常が存在するとき，どの器官がどんな場合も常に病んでいなければならないのか？——この問いに対する答えが精神医学総体の第一前提である．

この問題の器官が脳に他ならないことが，生理学および病理学の諸事実によって示されるならば，精神病には常に脳の疾患を認めなければならない．

各種の最新の脳計測機器や新しい医薬品や技術開発というその装いをはぎ取ってみれば，行動変容を目的として脳に神経科学的介入を行うという発想は，グリージンガーの延長線上にあって，取り立てて先端的とはいえない．その意味では，本章で行ったように，過去に論争となった事例の倫理学的な検討からは，多くを学ぶことができるのではないか．

本章では，神経科学的な脳への介入の臨床応用の現状を踏まえた上で，その倫理学的な問題点を考察した．だが，可能性として，将来の神経科学的介入が「今日の先進社会を成り立たせている基本的な前提の変更」を引き起こす「先端医療」となることは十分あり得る．例えば，本人が知らない間に（あるいは本人の意志に反して），人格や記憶を自由に操作できるようになれば，近代的自我が前提としてきた「人格の恒常性」が揺るがされ得る．また，心のなかの思考を本人が知らない間に（あるいは本人の意志に反して）読み取ること（マインドリーディング）が可能となれば，内心の自由というプライバシー権の社会的な扱いが大きく影響されるかもしれない．そうした可能性については，ニューロエシックス（脳神経倫理）の観点から改めて考察する必要があろう*74．

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【注】

*1 黒田浩一郎，2010「先端医療，先端性，社会学」佐藤純一ほか編『先端医療の社会学』世界思想社，2頁．

*2 もちろん，このことは，一般論として身体のほかの臓器にもあてはまる．例えば，のどにある扁桃腺は，かつては機能的に重要でない身体部分として，感染症による炎症があった場合に積極的に切除されていたが，こんにちでは免疫系の一部としての役割が解明されつつある．

*3 Definitions of SFN, World Society for Stereotactic and Functional Neurosurgery (http://www.wssfn.org/) (インターネット情報最終閲覧日→2012/6/16)

*4 Mashour, G. A. et al., 2005, "Psychosurgery: past, present, and future", Brain Res Rev vol. 48, pp.409-419.

*5 フォード，P. J.，ヘンダーソン，J. M.，2008「機能的脳外科手術の介入：手術室の中の脳神経倫理学」，イレス，J. 編『脳神経倫理学：理論・実践・政策上の諸問題』篠原出版新社，所収．Gildenberg, P. L. 2009, "History of Surgery for Movement Disorders", In Balay, R. A. E. (ed.) *Movement Disorder Surgery*, Thieme Medical Pub.

*6 Horsley, V. 1890, "Remarks on the surgery of the central nervous system", BMJ vol. 2, pp.1286-1292, Horslay, V. 1909, "The function of the so-called motor area of the brain", BMJ vol. 2, pp.125-132.

*7 Meyers, R. 1940, "Surgical procedure for postencephalitic tremor, with notes on the physiology of premotor fibers", Arch Neurol Psychiatry vol. 44, pp.455-459, Meyers, R. 1958, "Historical background and personal experiences in the surgical relief of hyperkinesia and hypertonus", In Fields, W.S. (ed.) *Pathogenesis and Treatment of Parkinsonism*, Charles C Thomas, pp.229-270.

*8 Horsley, V., Clarke, R.H. 1908, "The structure and function of cerebellum examined by a new method", Brain vol. 31, pp.45-124.

*9 Spiegel, E.A. et al., 1947, "Stereotaxic apparatus for operations on the human brain", Science vol. 106, pp.349-350.

*10 Meyers, R. 1951, "Surgical experiments in the therapy of certain 'extrapyramidal diseases'", Acta Psychiatr Neurol vol.26, pp.1-42., Spiegel, E.A. et al., 1958, "Long-range effects of electro-pallido-ansotomy in extrapyramidal and convulsive disorders", Neurology vol. 8, pp.734-740., Riechert, T. 1962, "Long-term follow-up of results of stereotaxic treatment of extrapyramidal disorders" Confinia Neurologica vol. 22, pp.356-363.

*11 Horsley, V. 1886, "Brain surgery", BMJ vol. 2, pp.670-675.

*12 Japer, H. and Penfield, W. 1954, *Epilepsy and the Functional Anatomy of the Human Brain. 2nd edition*, W. Little, Brown and Co.

*13 Leitinen, L.V. et al., 1992, "Leksell's posteroventral pallidotomy in the treatment of Parkinson's disease", J Neurosurg vol. 76, pp.53-61.

*14 Benabid, A.L. et al., 1987, "Combined (thalamotomy and stimulation) stereotactic surgery of the VIM thalamic nucleus for bilateral Parkinson disease", App Neurophysiol vol. 50, pp.344-346.

*15 Deuschl, G. et al., 2006, "Deep brain stimulation: postoperative issues", Mov Disord vol. 21 (Suppl 14), S219-237.

*16 Beijani, B. P. et al., 1999, "Transient acute depression induced by high frequency deep brain stimulation", NEJM vol. 341, p.1004.

*17 Wichmann, T. and DeLong, M. R. 2006, "Deep brain stimulation for neurologic and neuropsychiatric disorders", Neuron vol. 52, pp.197-204.

*18 ロボトミーは，ロイコトミー，ロベクトミーなどと並ぶ精神外科の手術方法の一つであるが，一般には精神外科手術の代名詞として使われている．

*19 日本語でのロボトミー論争に関する概要としては，栃島次郎，2008「精神外科と脳研究の過去と現在」『臨床評価』36巻，85-114頁，同上，2008「脳科学は『非侵襲的』たりうるか？」『現代思想』36巻，156-165頁．本稿脱稿後に，同上，2012「精神を切る手術」岩波書店が，出版された．邦訳では，L・スレイター，2005『心は実験できるか：20世紀心理学実験物語』（岩坂彰訳）紀伊國屋書店の第10章，W・フリーマンの伝記であるJ・エル＝ハイ，2009『ロボトミスト』（岩坂彰訳）ランダムハウス講談社が詳しい．英語では，Shutts, D. 1978: Lobotomy: *Resort to the knife*, Van Nostrand Reinhold Co., Valenstein, E.S. (ed.) 1980: *The psychosurgery debate: Scientific, legal and ethical perspective*, W.H.Freeman and Co., Valenstein, E.S. 1980: *Great and desperate cares:*

The rise and decline of psychosurgery and other radical treatments for mental illness, Basic Books, Pressman, J.D. 1998: *Last resort: Psychosurgery and the limits of medicine*, Cambridge University Press. などがある.

＊20 原作は，キージー，K.，1996『カッコーの巣の上で』(岩元巌訳)冨山房.

＊21 World Health Organization 1976: Health aspects of human rights. WHO.

＊22 Moniz, E. 1936, "Essai d'un traitement chirurgical de certaines psychoses", Bulletin de l'Academie de Medicine vol. 115, pp.385-392.

＊23 厳密には，最初はメスで脳内の神経経路を切断するのではなく，前頭葉に注射器でアルコールを注入して組織を破壊することで同じような効果を得ることを狙った.

＊24 Valenstein, 1980, *The psychosurgery debate* 所収の Chapter 8 Review of the literature on postoperative evaluation (Valenstein, E.S.), Chapter 11 The psychosurgery evaluation studies and their impact on the commission's report (Chorover, S.L.)に詳しい.

＊25 Swazey, J. 1974, *Chlorpromazine in psychiatry: A study of therapeutic innovation*, The MIT press.

＊26 Valenstein, 1980, *Great and desperate cares*, p.154.

＊27 エル＝ハイ，2009『ロボトミスト』.

＊28 Valenstein, 1980 *The psychosurgery debate*, pp.37-38 (Freeman, W. and Watts, J.W. 1944, "Physiological psychology" (Luck, J.M. and Hall, V.E. (ed.), Annual Review of Psychology, Vol 6 からの引用).

＊29 Shutts, 1978 *Lobotomy*, p.113.

＊30 例えば，フリーマンにロボトミーを受けたH・ダリーはその自伝のなかで，ロボトミーを受けたことでゾンビのような怪物扱いされることの苦痛を吐露している(ダリー，H.，フレミング，C.，2009『ぼくの脳を返して：ロボトミー手術に翻弄されたある少年の物語』(平林祥訳) WAVE 出版).

＊31 美馬達哉，2010『脳のエシックス』人文書院．生命環境倫理ドイツ情報センター，2007『エンハンスメント バイオテクノロジーによる人間改造と倫理』知泉書館.

＊32 モノアミン仮説とは，人間の脳内の神経細胞の間隙(シナプス)にあって情報伝達に関わっている神経伝達物質のなかでもモノアミンと総称される物質(ドパミン，セロトニン，ノルアドレナリンなど)が，情動や気分や意欲と密接に関連しているという仮説である．とくに，特定の神経細胞から放出されて別の神経細胞の受容体に結合するセロトニンとノルアドレナリンが減少すると，憂うつ感が引き起こされて，うつ病が生じると考えられている．そして，うつ状態に対して最近よく使用されるSSRIとは，この放出された神経伝達物質が元の神経細胞に再び吸収されることを防いで，シナプスでの神経伝達物質の量を増やして情報伝達を正常化するとされる．しかし，これはあくまで仮説であって，セロトニン調節作用が強いほど抗うつ効果が強いとは限らない事実も知られており，抗うつ薬の作用機序の詳細は解明されていない(ヴァレンスタイン，E. S.，2008『精神疾患は脳の病気か：向精神薬の科学と虚構』(切刀浩監訳)みすず書房，ヒーリー，D，2004『抗うつ薬の時代：うつ病治療薬の光と影』(林建郎，田島治訳)星和書店，カーシュ，I.，2010『抗うつ薬は本当に効くのか』(石黒千秋訳)エクスナレッジなど).

＊33 シェフ，T. J.，1979『狂気の烙印：精神病の社会学』(市川孝一，真田孝昭訳)誠信書房，サズ，T.，1975『精神医学の神話』(河合洋ほか訳)岩崎学術出版社，カチンス，H.，カーク，S. A.，2002『精神疾患はつくられる：DSM診断の罠』(高木俊介，塚本千秋訳)日本評論社など.

＊34 コンラッド，P.，シュナイダー，J. W.，2003『逸脱と医療化：悪から病いへ』(進藤雄三監訳)ミネルヴァ書房．Conrad, P., 2007, *The medicalization of society*, Johns Hopkins University Press.

＊35 Porsolt R. D. et al., 1977, "Behavioral despair in mice: a primary screening test for antidepressants" Arch Int Pharmacodyn Ther vol. 229, pp.327-336, Petit-Demouliere, B. et al., 2005, "Forced swimming test in mice: a review of antidepressant activity" Psychopharmacology vol. 177, pp.245-255.

＊36 ケストラー，A.，1995『機械の中の幽霊』(日高敏隆，長野敬訳)筑摩書房，39頁.

＊37 キージー，1996『カッコーの巣の上で』.

＊38 ヴァレンスタイン，2008『精神疾患は脳の病気か』など．本節の記載は，依存という観点から向精神薬の歴史を考察した拙論，2010「医療内アディクションと神経化学的自己」(『現代思想』38巻14号122-134頁)と一部重なっている.

＊39 ある種の物質(アヘン，酒など)が，人間の精神状態に影響することは古くから知られていた．鎮静効果のある医薬品として広く用いられてアヘン類は，一九世紀末ごろから麻薬として使用制限されるようになった(「万国アヘン条約」1912年).

＊40 Smith, M. C. 1991, *A social history of the minor tranquilizers: The quest of small comfort in the age of anxiety*, Haworth Medical Press.

＊41 乱用や依存が問題となって日本では1972年から医師の処方箋が必要となった．松枝亜希子，2010「トランキライザーの流行：市販向精神薬の規制の論拠と経過」Core Ethics vol. 6, pp.385-399.

＊42 Gabe, J., Bury, M. 1996, "Halcion nights: a sociological account of a medical controversy " Sociology vol. 30, pp.447-469., Abraham, J. and Sheppard, J. 1999, *The therapeutic nightmare: The battle over the world's most controversial sleeping pill*, Earthscan publications., メグワー，C.，ハートン，A.，2005，『暴走するクスリ：抗うつ剤と善意の陰謀』(吉田篤夫ほか訳) NPOJIP.

＊43 Swazey, 1974. *Chlorpromazine in psychiatry*

＊44 コンラッド，シュナイダー，2003『逸脱と医療化』Conrad，2007，*The medicalization of society*.

＊45 ヒーリー，D.，2004『抗うつ薬の時代』(林建郎ほか訳)星和書店.

＊46 クレイマー，P. D.，1997『驚異の脳内薬品』(堀たほ子訳)同朋社，ヒーリー，D.，2005『抗うつ薬の功罪：SSRI論争と訴訟』(田島修監修，谷垣暁美訳)みすず書房.

＊47 Teicher, M. H. et al., 1990, "Emergence of intense suicidal preoccupation during fluoxetine treatment", Am J Psychiatr vol. 147, pp.207-210.

＊48 ヒーリー，D.，2005『抗うつ薬の功罪』.

＊49 イギリスでの論争の経過についてはメグワー，ハートン，2005『暴走するクスリ』.

＊50 美馬達哉，2010「医療内アディクションと神経化学的自己」.

＊51 冨高辰一郎，2010『なぜうつ病の人が増えたのか』幻冬舎ルネッサンス新書.

＊52 モイニハン，R.，カッセルズ，A.，2006『怖くて飲めない 薬を売るために病気はつくられる』(古川奈々子訳)ヴィレッジブックス，エンジェル，M.，2005『ビッグファーマ 製薬会社の真実』(栗原千絵子，斉尾武郎訳)篠原出版新社.

＊53 ECTの歴史については，Shorter, E. and Healy D., 2007, Shock Therapy: A history of electroconvulsive treatment in mental illness, Rutgers University Press. が詳しい．ECTを治療目的で推進する立場から倫理原則を述べたものとして，Ottoson, J-O.,

Fink, M., 2006『電気けいれん療法の実践的倫理』(中村満訳)星和書店がある．日本での状況の紹介として，吉村夕里，2007「精神医療論争　電気ショックをめぐる攻防」Core Ethics vol. 3, pp.375-390 がある．

＊54 Cerletti, U., 1940, "L'Elettroshock", Rivista Sperimentale di Frenatria vol. I, pp.209-310.

＊55 水野昭夫，2007『脳電気ショックの恐怖再び』現代書館，コット，J.，2007『奪われた記憶　記憶と忘却への旅』(鈴木晶訳)求龍堂．

＊56 2002『米国精神医学会タスクフォースレポート：ECT実践ガイド』(日本精神神経学会電気けいれん療法の手技と適応基準の検討小委員会監訳)医学書院．

＊57 Sakel, M. 1934, "Schizophreniebehandlung mittels Insulin-Hypoglykamie sowie hypoglykamischer Schocks". Wiener Medizinische Wochenschrift vol. 84, p.1211.

＊58 Meduna, L., 1935, "Versuche über die biologische Beeinflussung des Ablaufes der Schizophrenie. I. Campher und CardiazoleKrämpfe", Z Ges Neurol Psychiat vol. 152, pp.235-262.

＊59 Nuttin, B. et al., 1999, "Electrical stimulation in the anterior limbs of internal capsule in patients with obsessive-compulsive disorder", Lancet vol. 354, p.1526.

＊60 Mayberg, H. S. et al., 2005, "Deep brain stimulation for treatment-resistant depression", Neuron vol. 45, pp.651-660.

＊61 Penry, J. K., Dean, J. C. 1990, "Prevention of intractable partial seizures by intermittent vagal stimulation in humans: preliminary results", Epilepsy vol.31, S40-43.

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特集 II 尊厳死は誰のものか

尊厳死論を超える

緩和ケア、難病ケアの視座

中島 孝

医療アウトカムの混乱

現代では、あらゆる患者が外来や救急を通して病院に集まり、病院は悲鳴をあげていると言われている。けっして治癒することのない、慢性疾患患者、難病患者、認知症患者、治らないがん患者、超高齢障害者などがその多数を占めている。治せない病気を治して欲しいと依頼された病院は苦悩し、限られた資源で運用されている医療は疲弊し、質が低下し、混乱が起きているとされている。この問題を解決する為に、社会は、どのくらいの人や費用を医療に分配すれば良いのか分からないまま、「高齢者医療」「難病医療・緩和医療」、「終末期医療」の現場で、多様な問題が顕在化し、先鋭化している。一般的には、現代医療の進歩、特に延命治療技術の進歩と社会の超高齢化がこの問題の原因と言われている。しかし、実は、この考え方は間違っているばかりか、自ら解決の道を閉すという、二重の誤りをおかしている。

医療により人は「健康」になれるという幻想の下で、「健康」を増進するために作られた地域保健医療計画と診療報酬体系のパラダイムによって、あらゆる人々が無意識的に、一律に病院に導かれているのである。そこで、多数を占めている治癒を目指せない患者に対して、数としては少数でしかない治る患者向けの医療モデルが標準適用され、平均在院日数の短縮を目標に医療がおこなわれている。治る患者を早く治し、治らない患者は早く医療の領域から出すことが、理想の医療の姿となっているが、治せる患者が極めてすくないため、うまくいかず、ひどい混乱となっている。現代日本の医療システムは他国のモデルを表面的に導入してきたが、このような問題に対して、自ら解決するための研究をアカデミアも研究所も行なってこなかった。

さらに悪い事は、現代医療を改善するための科学的方法と呼ばれるものには、認識論上の問題があって、かえって混乱をひきおこし、医療カタストロフィーをむしろ悪化させている。医療経済論議で使われる「効率性」は、追求すればするほど医療現場の混乱を招いている。効率性を論じているはずの病院経営管理指標や機能評価では、評価対象、すなわち分子として費用で割られるものが本来何であるのか一切定義せず、代理指標（サロゲート）のみが提示され、医療の意味、真の医療アウトカムは論じられていないからである。

この様な状況においても、保健医療従事者は、治癒を目指せなくても患者のQuality of Life（QOL：生活の質）は高められると信じ努力している。しかし、どんなにがんばっても治らず、症状が良くならず、QOLすら悪化し続ける患者に直面すると、自分の仕事の評価ができなくなるばかりか、意味すら分からなくなる。「治らない患者をケアし続けることは医療の無駄ではないか」「この患者はどうせ死ぬのだから本人が希望すれば、無駄な延命はせず、早く死ぬ方が良いのではないか」「QOLが向上しないなら、死なせてあげたほうが良いのではないか」と葛藤することになる。誠実で真面目な医師や看護師ほど、解決できない課題によって自ら燃え尽きることになる。ここに「尊厳死問題」に医療従事者が関心を寄せる理由がある。しかし、そこには、医療におけるQOL概念の誤解がある上、真の医療アウトカムや意味を考えないまま、仕事をしている現場の混乱がある(1)(2)。

私が、八年前に副院長として病院に赴任した際に直面した問題はまさにこれであり、病床利用率は低いにもかかわらず、看護師など医療スタッフは重症者の対応で慢性的に疲れ切り対立しあい、管理職は患者の満足度を高める方法も、医療内容を改善する方法も分からないまま、看護職員が毎年、多数辞職する悪循環におちいっていた。

医療における健康概念、アウトカム概念を見直し、ケア方法の理論的再構成をおこない、この問題を解決する実践モデルを確立することで、職員と患者の笑顔と共に医療を再生することができた。問題の源泉は「尊厳死」ではなかった。現代医学における科学的方法のほとんどは似非科学でしかなく、現代医療は科学の名の下で、主観と客観を逆転させるという間違った歩みをしていたことが原因だった。この倒立状態の再逆転さえできれば、医療は真の科学性を取り戻せると考えた。

現代医療のイノベーション

いわゆる科学的な現代医療モデルとは根拠に基づく医療（EBM：Evidence based medicine）のことであり、すなわち、臨床試験（治験）により何らかの臨床結果（アウトカム）の改善が確率論的に証明された治療法を、患者はインフォームド・コンセントの下で自己決定し、多専門職種（multidisciplinary team）が協働し、クリティカルパス（critical path）により、最短経路で安全かつ効率的に実施するものとされる。

現代医療は、このモデルで解決できない問題に直面すると、医療費の分配の問題にすり変えるか、倫理委員会に投げかけるか、法的問題にしようとするが、それらは完全に誤りである(3)。本来はこの医療モデル自体を科学的に変えて、問題が起きない構造にすべき

学療法、外科手術、放射線療法、胃瘻造設術、人工呼吸器療法も緩和になるのである。日本の緩和医療制度では残念ながら、そのようになっていないが、本来のこの緩和ケアを行えれば、がんはもちろん、慢性疾患や認知症、あらゆる治癒を目指せない疾患に対しても、災害時においても、ケアの質が改善し、患者と家族、保健医療従事者だけでなく、地域社会全体に笑顔がもどり、人は世代を交代しながらも、肯定的に生きて行けることは明白なのである。

英国の緩和ケア運動は全世界に広がったが、WHOは緩和ケアを、An approach that improves the quality of life of patients and their families facing problems associated with life-threatening illness…（人生を脅かす疾患による問題に直面している患者と家族のQOLを改善するアプローチである）。と定義した（二〇〇二年）。緩和を患者・家族のQOLの向上としたため、QOL概念を誤解すると緩和ケアも同時に理解できなくなる問題がおきている。不幸なことに、現代において一般的に誤解されているQOL概念によって「緩和ケアとは痛みをコントロールし、QOLが向上できない場合に、無駄な延命をせず死を受容するためのケア」と誤解されることになった。このため、QOL概念の誤解を解く必要がある。

誤解された尊厳

QOLは、緩和ケアのみならず、様々な領域に使われているが、意味も計量性についても完全に誤解されている[5]。医療でQOLの向上という際には、何らかの手法により順位付けるか、計量可能な科学概念と考えられ、「生活の質」と翻訳される。一方、「生命の尊厳（SOL：Sanctity of Life）」の尊厳は、科学的評価・測定が不能な対象に対する哲学概念であり、宗教的な接点も持つ。「人自体の価値は計量できず、人は相互に置き換えられない存在」という意味が尊厳なのである。しかし、両者を混同し、SOLの代理指標をQOLと誤解すると、QOLが低い人は尊厳が少ないので生きる価値が少ないと誤って考えてしまう。一方、「人の命の質は計られるものでなく、良い命とか悪い命とかいう評価はできない」という文脈での「命の質」は実は、QOLではなく、尊厳（SOL）のことである。しかし、QOLを間違って「命の質」と訳す誤解が絶えないのは残念である。この様な尊厳概念の誤解によって、「尊厳ある死」は医学的、客観的に定義でき、計画的に実行できるとする誤解が生まれ、一般市民だけでなく、医師、政治家、法学者も混乱することになった。

効用＝QOLの誤解

厚生経済学は社会全体が幸福になるための所得の分配を研究する学問であり、ベンサムが功利主義で論じた「最大多数の最大幸福」を実現するために、個人の幸福、福利を効用値（utility）として計量しようとする。そして、効用値を算出する際に、健康な生を1、死を0とし、ゲーム理論に由来する期待効用理論を使うことでおかしな理論となった[6]。まず、健康とはなにかが問題なのだが、その前に、期待効用理論は計量心理学的に正しいかという方法論的問題が注目された。

その解決のため、国民データから計量心理学的に標準化された効

だからである。しかし、現代ではこのモデルを盲信し、「健康」を目指す医療をおこなうことが前提とされているため、まじめに行うほど、治癒を目指せず、健康になれない患者の医療は空回りとなり、医療の無駄（medical futility）を生み出していると思われることになる。その場合にこそ適切な医療が必要なのでどうすればいいか分からず混乱してしまうことになる。「健康概念」に基づくアウトカム評価によれば、どんな熱心な医療によっても、これらの患者は常に悪化するだけなのである。これは、「健康概念」自体がおかしいからおきるのだが、ほとんどの人はそれに気がつかず、その様な治らない患者は十分に説明を受け「死を選ぶ」か「延命治療を選ぶ」かを自己決定すべきで、自己決定能力がなくなる前に、事前にそれを自分の選好により決定するのが良いという考えが論理的に正しいとされてしまう。

人は本来、自ら人生を放棄したり、死を自己決定したりする必要はなく、人は誰でも死を迎えるまで活き活きと生きることが可能なはずである。そのための医療は工夫すれば可能なはずなのである。実は、この問題を解決するために医療でイノベーションされたものが、一九六七年に英国のロンドン郊外シデナムで生まれた緩和ケアであり、それは今でも、現代の医療問題を根本から解決する力をもっている。しかし、この緩和ケアは日本を含む全世界で誤解され続けており、我々は混乱の中にいる[4]。

本来の緩和ケア運動

日本の緩和医療制度では、医療内容は実質的に制限され、対象患者は「がん」にほぼ限られ、本来の緩和ケアとはまったく違うものになってしまった。一方、緩和ケア発祥の英国ホスピスや英国在宅ケアでは、小児領域や難病を含むあらゆる治らない病態が緩和ケアの対象となっている[3]。シデナムのセントクリストファーホスピスの創立者であるシシリーソンダースは「人は治療困難な病気に直面した時、無駄な治療を続け苦痛に満ち希望のない療養をすべきか？『死を受容』し、あきらめて死を早く迎えるべきか？苦悩する問題に着目し、この苦悩に対してどのようなケアを行えば解決できるのか」を考えた。彼女は、治療概念をやめ枠組みを変え、全人的苦痛（トータルペイン）に対する緩和概念に変えればこの問題を一気に解決できると考えた。人は健康な人も、病気の方もすべて最終的に、治らない病気になって死ぬ。もともと生命は潜伏期間や発症時期は不明だが、すべて致死率一〇〇パーセントの難病に一〇〇パーセント罹患するのである。このため、治らない病気になるなら、生きることをあきらめる（abandon life）という考えはおかしく、どんな場合でも死を迎えるまで、全人的苦痛の緩和をおこなうケアを続けられれば生を肯定して生きられる（affirm life）はずだと考えた。多専門職種（multidisciplinary team）のケアにより、症状がコントロールされ、身体的、心理・社会的な苦悩が除去されると、患者、家族は、死を受容するか否かではなく、治らない病気、死に至る病気と共に生きる人生であっても、再び肯定し活き活きと生きられるようになるのである。そして、死別したあとも、残された人は、悲しみから再生し生きて行くことを支援されるのである。医療技術は全人的ケアの文脈で使われるなら「単なる延命治療」ではなくなり、化

(the ability to adapt and self manage in the face of social, physical, and emotional challenges)という考えで健康をとらえようとしている(7)。この定義であれば、正常状態に、または健康状態に戻す事が医療なのではなく、問題に対する適応能力を改善したり、補ったりすることが医療の目的となり、治癒を目指せない疾患の医療は理論的な破綻なく構成できるようになる。これにより、「健康になれない人、治らない人への医療は本来不要なのだ」という医療の無駄論議にも終止符をうつことができる。

客観から主観へ

本来の医療の目的は人が主観的に改善することであり、客観指標は単なる代理指標にすぎないのだが、現代医学においては戦前のドイツ医学由来の伝統から、あらゆる国で、患者の主観よりも、客観評価の重視が当たり前となった。患者の主観評価、自己評価は心理状態によって変化するため、信用できず、科学評価に値しないと思われた。そのため、現代医学は客観指標にのみ基づいて行われ、患者のナラティブ(narrative)を聞くのは付け足しでしかなく、画像や検査データに基づいて医療は進められている。しかし、二〇〇〇年代に入り、臨床試験(治験)で、介入や治療効果の評価指標として、主観評価として「患者の報告するアウトカム(PRO:patient reported outcome)」評価が提唱され、QOLもPROの一つとして概念整理された。現代医療において、患者の主観評価はようやく復権したのだが、治癒を目標にできない領域ではとりわけPROは重要になった(5)。

PROは患者の主観的評価を自己記入式や面接法によりおこなうもので、症状、機能、健康状態、QOL、医療内容などあらゆるものが評価対象となる。QOL(生活の質)はPROであり、患者の人生・生活に対する主観的評価であり、他者が判断する「人間らしさ」の評価ではない。現在、PROは医薬品や医療機器の治験・臨床試験における評価項目として重視されている。

レスポンスシフトの評価

現代医療が患者のナラティブを聞かなかったのは、患者の主観評価を科学的に扱う際に問題となるレスポンスシフト(反応シフト:response shift)現象の研究が不十分だったからである。人は同じ事象に対しても、時間や状況が異なれば、異なった主観評価をする特徴を持つ。それをレスポンスシフト現象と呼ぶ(5)(6)(8)。代表例は薬理反応試験におけるプラセーボ効果である。時と場合により、患者自身の内的な評価尺度が変わるためにおきるが、生活分野の重みづけが変化したり、痛みの閾値が変わったり、再解釈のためにおきる。レスポンスシフトが科学的に解明されるにつれて、PROは臨床評価として使える様になった。PROを臨床評価として使うことで、改善効果だけでなく、良いレスポンスシフトをおこす治療か否かも分かる様になった。本来、医療・ケア技術の善し悪しは主観や客観による介入評価だけではなく、人に良いレスポンスシフトをひき起こせるかどうかも評価すべきなのである(5)。

SEIQoL:The Schedule for the Evaluation of Individual Quality of Life(個人の生活の質評価法)はアイルランドのダブリンの王立外

用価を算出するために開発されたのが、EuroQoL(EQ-5D)であり、そこでは効用値とQOLは同じとみなされている。EQ-5Dでは事前に決められた健康に関する五分野を人が自ら三段階評価し、標準化された変換テーブルを参照し効用値を算出する。五分野すべてを最低評価とすると、効用値は0(=死)以下となる。これは、国民データは健康集団であり、重篤な病気を不安に感じ、死より悪いと考え、忌避したいという意識を持っていることを反映している。この効用値は健康集団の集合意識を統計学的に標準化したのであり、病気の方の健康意識は反映されない。このため、治癒を目指せない患者のアウトカム評価には本来使えない。しかし、このモデルを使い、医療費の支払いモデルをつくろうという誘惑が歴史上絶えたことがない。効用値の低い人には医療費の支払いを少なくするという、効用値を利用した質調整年(QALY:Quality adjusted life year)の利用はオバマ大統領が二〇一〇年に行った医療保険改革の「患者保護と入手可能なケア法(Patient Protection and Affordable Care Act)」中で禁止されたが(5)、我が国では最近評価される逆転がおきている。注意すべき点は、この効用値を人の存在価値、尊厳の代理指標と誤解し、医療費分配に使うと、ナチのホロコーストの理論である「Lebensunwertes Leben(生きるに値しない生命)理論」と差がなくなり、大変危険である。

健康関連QOLの問題

医療におけるQOL評価は、健康関連QOL評価(HR-QOL:Health related QOL)が標準とされている。代表的なHR-QOL評価尺度におけるSF-36の八領域、EQ-5Dの五領域はいずれもWHOの健康概念「健康状態とは、身体的、精神的および社会的に完全に良好であること(well-being)であり、単に病気や病弱ではないことではない」(一九四八年世界保健機関憲章前文)に由来し、計量心理学的な検討を経て作られたもので、それらを患者が主観評価することをQOL評価としている。治癒し得ない進行性の疾患では、いくら治療しケア介入しても、客観的な「健康状態」になれないが、健康概念を使う限り、HR-QOL評価でも同様である。治らない病気では治療やケア効果の評価として、HR-QOL評価尺度を使う妥当性がない。HR-QOLが低い患者ほど、医療が必要であることは明白であるがその効果は示しえないのである。難病ケアや緩和ケアの目標はQOLの向上だが、HR-QOL評価尺度では決して医療による介入効果を示しえないことを第一に科学的に理解しておく必要がある(6)。

健康概念の見直し

あらゆる人は、不定の時期にだが、必ず死ぬのであり、その過程で、すべての人が治らない疾患になる。このため、国民全体、全年齢、全疾患を対象とする保健医療福祉政策を立案するためには、この問題に対応できていないWHOの健康概念からできる限り早く、抜け出し、治癒しない疾患にも対応できる健康概念を確立する必要がある。二〇一一年にBMJ(British Medical Journal)で提案された新しい健康概念はWHOの健康概念を変え、新たに、社会的、身体的、感情的な問題に直面した際の適応能力や自己を管理する能力

心を思いやりながら工夫し、知らせる過程で医療従事者は患者から信頼を得るのである。予測できない、不可逆的に悪化して行く病態に対して適切な援助を継続するためには患者・家族との信頼関係が必要であるから重要なのである。告知の第一目的は、「死の受容」や「疾患の受容」ではなく信頼関係のもとで、人は生きている限り適切なケアが受けられ、幸福に過ごせるはずだという価値の共有にある。

医療における受容理論は、障害受容概念として、一九五〇年にGraysonが提唱した[9]。ステージ理論は社会科学や力動的心理学の影響により提唱されたが、そこでは受容概念ではなく、最初は、適応概念だった。しかし、一九六九年、Kübler-Rossは『死ぬ瞬間――死にゆく人々との対話』において、ステージ理論と受容理論を合わせたモデルを提唱した[10]。これは否認、怒り、取引、抑鬱、受容の五段階を経て人は希望に至るという心理モデルであり、世界に大きな影響を与えた。この影響の下で、一九八〇年、リハビリテーション医学指導者の上田敏は「障害受容」とは「あきらめ」でも「居直り」でもなく価値転換であるとし、患者が障害や疾患の受容に至ることがリハビリテーションや治療に必須であるとした[11]。日本では緩和医療で、この受容理論を導入した。しかし、個別ケアにこのKübler-Rossの受容理論を当てはめれば当てはめるほど、受容できない患者に無理に受容を促すだけで、患者のナラティブをケアチームが傾聴しなくなり、受容できない患者とレッテルを貼ることで、関係性が崩れ、その結果、患者は自らを言語化し再構成して生きて行くことがむずかしくなるという問題がおきた。本来、価値観の転換（ギアチェンジ）は患者に要求するものではなく、医療従事者がまずする必要があるものなのである。

英国緩和ケアではもとよりこの理論は使わなかったし、この理論の科学性への疑問、疾患受容の行動化の困難、障害受容に至らない人への蔑視がおきる悪影響があるために廃れた。それではこの領域ではどのようなアプローチが有効なのだろうか。

ナラティブアプローチ

紀元前五〇〇年頃のインドで、シッダールタは、人は老い、病み、死ぬことで喪失することを思い悩んだ。自分も老い、病み、死するものにかかわらず、他人の老い、病い、死を嫌悪し、自分はそれらの運命から自由になれる様に思うが、実際にはこの運命からだれも自由ではないはずで、どうしてこんな心の矛盾を自分は持って生きているのだろうかとさらに悩んだ。人間は生きる中で、物事の認識、解釈をつねに変えながら、世界を再解釈し、この矛盾に対応し日々を過ごしているが、これがナラティブアプローチのはじまりであるといわれている[13]。

一九九〇年、構成主義（constructivism）の影響の下でエプストンとホワイトは精神科家族療法を改善するために、『Narrative means to therapeutic ends（物語としての家族）』で、ナラティブ療法を発表した[14]。解決困難な問題とはドミナント・ストーリー（主要なストーリー）が十分に生きられた経験を表していない状態ととらえる。汲み残された経験に光があたると、オールターナティブ・ストーリー（もう一つの代替ストーリー）が創生される。問題は患者個人や家族・

科大学病院のオ・ボイル教授らにより、従来の健康概念の縛りがないQOL評価尺度として作られたが、レスポンスシフト現象も科学的に評価できる方法として高く評価されている[5][6][8]。これはQOL評価尺度であるがPRO評価の代表であり、緩和ケア領域や難病ケア領域においてケアによるPRO／QOLの改善を評価できると考えられている。SEIQoLは半構造化面接法によりおこなうものである。患者の最も大切に考えている生活の領域を五個引き出し、患者自身により内容を定義してもらい、それぞれ名付けてもらう。次にそれぞれの生活領域がどの程度うまくいっているか／満足しているかをVAS（例、0-10）により評価してもらう。次に、五個のそれぞれの領域がその人の生活においてどのような重みで意識されているかを計測する。重みが分かれば、一次元的なQOLとしてSEIQoL-indexが算出できる。重みを計測するためには原法では判断分析法という多変量解析を利用する。この場合、妥当性評価と信頼性評価が同時に数値的に算出される。この方法では時間がかかるため、実際の臨床場面では直接的に重みづける方法（DW法：direct weighting法）が頻用される。被検者にパイチャートに類似した円盤を操作してもらい、重みを直接表してもらう方法である。

治癒を目指せない進行性の疾患では、人は症状の変化に応じて、自分自身の生活領域の重要度を変化させながら生きている。健康関連QOL評価尺度は生活領域が固定されているために、その人にとってすでに無関係な生活領域を評価対象とする場合がある。SEIQoLは動的に生活領域が変化している状態でも評価可能であり、計量心理学的にも正しい評価方法である[1][3][6]。

喪失を捉え直す

実際のケアにおいてこれらの理論を実践するためにはまず、治癒しないということに人はどう向き合うかを研究する必要がある。人は喪失（loss）の中にあってもどのように生き、サポートしあうのかというテーマは悲嘆ケアとして研究されてきたが、今まで、愛する人の喪失すなわち死別（bereavement）研究がほとんどだった。実際の喪失研究は、人のあらゆる喪失に対して行われるべきである。身体機能の喪失、治癒しない病気、老化、死という喪失があるし、災害の中での人命、財産、社会機能の喪失もある。個人が喪失から再生に向かうだけでなく、社会や世代が喪失から再生に向かうという普遍的なテーマを地域や時代を超えて、医療機関、福祉施設、地域社会でどのように科学的にアプローチできるのかどうかが現代の喪失研究であり、その中で医療は病気、老化、死をあつかうため大きな役割を担わされている。

キューブラーロスを越える

我が国では、喪失研究において、ステージ理論や受容理論が多く使われているが、それが現代医療の混乱の原因となっている。たとえば、治らない病気を患者・家族に知らせること、告知（breaking the news）の目的を「治らないことを伝え、死を受容させる」、「生きることを諦めさせるギアチェンジ」と誤解している医師、看護師が多い。本来、緩和ケア、難病ケアにおける告知の目的は、患者との信頼関係を作ることにある。説明しづらい良くない話を相手の

従事者が持っていた力や現代の医療技術は再び意味付けられ、医療はたちまち改善する。医療は人や社会を自律的に成長させ再生する力として再評価され、「尊厳死」問題は消失するだろう。同時に、経済学者のハイエクが「設計主義の誤り」と言った様に、高齢化社会において、効率良く尊厳死するために必要な合理的医療の制度設計を議論する厚生経済学はもはや不要となるだろう。その時には医療における分配や価格の問題も自然に解決されると思われる。このパラダイムチェンジには教育や研究やグループワークは必要だが、基本的に追加の費用はかからないはずである。

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介護者の属性として存在しているのではない。問題が外在化できると、新たな意味を見いだしながら、患者自身はストーリーを書き換え生きて行くことができるようになると考えた。これは治らない病気と共に生きる生活を支援する方法としても有効な方法といえる。

一九九九年にGreenhalghらはプライマリケア領域で「ナラティブに基づく医療（NBM：narrative based medicine）」を提唱した(15)(16)。診療を改善するためには、患者と医療チームとの心理的な協力関係の促進のためだけでなく、診断、治療のために、西洋医学の中で失われてしまった、患者の語るナラティブの意味を再構成する技術が必要であるとした。

二〇〇一年に、ミーマイヤらは、『喪失と悲嘆の心理療法――構成主義からみた意味の探究（Meaning Reconstruction and the Experience of Loss）』を発表した(17)。治らない疾患や死別における悲嘆ケアとしてはステージ理論や受容理論は有用ではなく、患者のナラティブから、意味の再構成を行いながら心理支援して行く方法が重要であるとした。

二〇〇九年にオリビエらは『保健医療におけるナラティブと物語（Narrative and stories in health care）』で、シシリーソンダースが英国緩和ケアで行ってきた基本的な方法はナラティブアプローチであることを表明した(18)。

ナラティブアプローチは治癒を目指せない緩和ケアや難病ケアの領域で医療を成功に導く支援方法であり、ステージ理論や受容理論の問題点を乗り越え、患者・家族・地域社会を支援することができる。このため、現代医療の必須技術になった。しかし、未だに医学や看護学の標準的教育カリキュラムに組み込まれておらず問題である。

変わり続ける人・社会

医療は本来患者の報告するアウトカム（PRO）を改善するべきだが、現代医学では代理指標でしかない客観的エビデンスを尊重する逆転がおきた。医師や看護師は客観指標のみを意識し、患者の主観的な改善感やナラティブを重視しなくなった。そのため、患者が生きて行く内的な力は奪われてしまった。これが現代の医療問題の根本原因である。この問題を解決するためには、PROを科学的にアプローチし、最初から、患者と関係者（家族など）の主観的な満足度を高めるための医療を提供する医学に作り替える必要がある。

この新しい流れを確立できれば、がん、非がん、難病、認知症、生活習慣病、高齢者医療、post-NICU医療、障害児（者）医療、小児慢性疾患医療、救急医療などすべてが同じ基盤で、地域で行うことができると考えられる。適切なケアさえあれば、どんな病気であっても、患者や関係者は生きる事をあきらめるのではなく、治らない病気と共に人生を肯定して生きられる様になる。どんな年齢であっても、どんな病気と共にあっても、たとえ意識障害が合併しているとしても、人は死を迎えるまで、変化し成長発達することができる。残された人々は喪失を乗り越え、継承し発展させられる。これが本当の自律概念であり、本来の緩和ケア、すなわち喪失から再生へのケアであり、地域を再生させる力となる。

この様に医療を再構成（reconstruct）するだけで、もともと医療

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ORIGINAL ARTICLE

Reduction rate of body mass index predicts prognosis for survival in amyotrophic lateral sclerosis: A multicenter study in Japan

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Abstract

Malnutrition in the early stage has been reported as an independent predictor of survival in amyotrophic lateral sclerosis (ALS). We analyzed retrospectively the effect of variation of body mass index (BMI) on survival in ALS patients. In total, 77 consecutive ALS patients were enrolled from nine hospitals in Japan. Reduction rate of BMI was calculated from BMI before the disease onset and at the time of the first visit to each hospital. We analyzed the correlation between BMI reduction rate and total disease duration. Results showed that the median BMI reduction rate was 2.5 per year (interquartile range 1.3–3.8). The BMI reduction rate was significantly correlated with survival length ($p < 0.0001$). There was also a significant difference in survival between ALS patients with a BMI reduction rate ≥ and < 2.5 (Kaplan-Meier survival analysis and the log-rank test, $p < 0.0001$; hazard ratio by the Cox model, 2.9816). In conclusion, faster reduction of BMI at the initial stage before the first visit to hospital predicts shorter survival length also in Japanese ALS patients.

Key words: *Amyotrophic lateral sclerosis, body mass index, malnutrition, survival prognosis*

Introduction

Patients with amyotrophic lateral sclerosis (ALS) often exhibit marked weight loss during the initial stage of the disease (1). Malnutrition has been reported as an independent prognostic factor for survival in ALS (1–3). Although previous studies have emphasized nutritional status at the time of diagnosis or gastrostomy, the relationship of initial BMI reduction rate before the time of diagnosis with survival prognosis has not been elucidated. In this study, we focused on the nutritional variation before the first visit, and analyzed its relationship to survival in ALS, as a first multicenter retrospective study in Japan.

Patients and methods

This study was performed as joint research among multiple neurology centers in Japan. Seventy-seven consecutive patients with sporadic ALS reaching the set endpoints (45 females and 32 males) were enrolled from December 2008 to November 2010. The endpoint was defined as the time when the patients died or were placed on ventilator.

Diagnosis of ALS was based on the revised El Escorial criteria (for more than possible ALS). We also included patients with progressive muscular atrophy (PMA) showing progressive deterioration up to bulbar palsy and respiratory failure. The body region of disease onset was evaluated—upper limb, lower limb, bulbar, and respiratory. Total disease duration was calculated as the interval from disease onset to the endpoint. Onset was defined as the time when the first symptom was noticed by the patients.

Each institute reported the following patient data: gender, onset date, onset age, onset body region, height (cm), weight (kg) before disease onset and at the time of the first visit, and the endpoint date. The weight before onset was based on declaration by patients. Values of arterial oxygen (PaO_2, mmHg) and carbon dioxide pressures ($PaCO_2$, mmHg), and forced vital capacity (% of the predicted value, %FVC) were also reported. Techniques of the measurements depended on the individual hospitals. Body mass index (BMI) was calculated (weight/height^2), and the cut-off value for malnutrition was set at BMI of 18.5 (1,4). We particularly focused on the BMI reduction rate (BMI-RR) from onset to the first visit, using the following formula: (BMI before onset–BMI at first visit)/(time interval from onset to first visit (y)).

We analyzed the correlation between total disease duration and the parameters at the first visit or BMI-RR (Spearman's rank correlation test). Subsequently, we compared the survival function among subgroups for each parameter using the Kaplan-Meier method and the log-rank test. We classified the patients by one-point BMI at the first visit (< or ≥18.5) and also by BMI-RR (< or ≥2.5, the median value). Univariate and multivariate analyses for survival were performed using the Cox proportional hazard model. $p < 0.05$ was considered significant.

Results

Median age at disease onset was 66.4 years (45–81 years). Onset regions were upper limb in 32 patients, lower limb in 23, bulbar in 20, and respiratory in two. Median total disease duration was 2.1 years (interquartile range (IQR) 1.4–3.2 years). Median BMI before disease onset and at the first visit was 22.9 (IQR 20.9–25.1) and 19.9 kg/m^2 (IQR 17.9–22.2), respectively. The median values of the BMI-RR were 2.5 kg/m^2/y (IQR 1.3–3.8).

There was no significant correlation between the total disease duration and the one-point values of BMI, %FVC, PaO_2 and $PaCO_2$ at the first visit. However, BMI-RR and the total disease duration showed a statistically significant negative correlation (Spearman's $r = -0.5409$, $p < 0.0001$; Figure 1). Comparison of the survival curves between the patients with BMI ≥ and <18.5 at the first visit showed no significant differences (log-rank test, $p = 0.5860$). Meanwhile, there was a significant difference in the survival curves between the patients with BMI-RR < and ≥2.5 (log-rank test, $p < 0.0001$, hazard ratio 2.535 (95% CI 2.068–6.058)) (Figure 2).

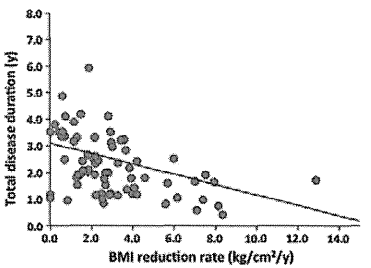

Figure 1. Correlation between the reduction rate of body mass index and total disease duration (Spearman's rank correlation test; $y = -0.1938x + 3.1054$, $r = -0.5433$, $p < 0.0001$).

Univariate analysis for survival by the Cox model showed a statistically significant effect on survival by BMI-RR (Table I). There were also weaker significant effects on survival by onset age and %FVC. Multivariate analysis for survival by the Cox model showed that BMI-RR and %FVC showed significant effects on survival (Table I). However, there was no significant correlation between the BMI-RR and %FVC.

Discussion

This study showed that the BMI reduction rate at the initial stage predicted survival in ALS. Consistent with previous reports (1–3,5), weight reduction or malnutrition proved an independent prognostic

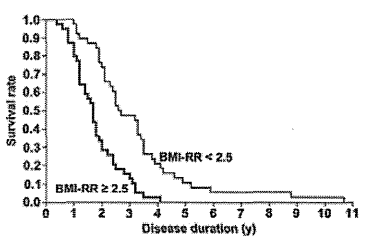

Figure 2. Comparison of the survival rate between amyotrophic lateral sclerosis patients with body mass index reduction rate (BMI-RR) lower and higher than 2.5 (log-rank test, $p < 0.0001$, hazard ratio 2.535 (95% CI 2.068–6.058)).

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Table I. Univariate and multivariate analyses for survival by the Cox proportional hazard model.

		Hazard ratio	95% CI	*p*-value
Univariate analysis				
BMI-RR	lower vs. higher than 2.5	2.982	1.820–4.930	<0.0001
Gender	male vs. female	0.888	0.563–1.416	0.613
Onset age	lower vs. higher than 65	1.632	1.015–2.681	0.043
Onset symptom	bulbar vs. non-bulbar	0.952	0.562–1.051	0.847
%FVC	higher vs. lower than 70	1.838	1.037–3.358	0.037
PCO_2	lower vs. higher than 45	0.905	0.483–1.647	0.748
Multivariate analysis				
BMI-RR	lower vs. higher than 2.5	2.741	1.470–5.126	0.001
Onset age	lower vs. higher than 65	1.019	0.541–1.994	0.955
%FVC	higher vs. lower than 70	1.928	1.054–3.640	0.033

BMI-RR: body mass index-reduction rate; %FVC: % forced vital capacity; $PaCO_2$: arterial carbon dioxide pressure.

factor in ALS also in Japan, indicating no racial differences. In addition, Figure 1 indicates that the greater the BMI reduction rate the faster the disease progressed, which might reinforce the importance of early nutritional intervention.

Since the first visit did not indicate the time of diagnosis in this study, BMI at the first visit varied among the hospitals. This might have caused the lack of significance in survival analysis when using the one-point BMI. Nevertheless, the survival analysis showed remarkably significant differences when using BMI-RR. BMI-RR reflects early-stage chronological nutritional variation before the first visit, and might be a better predictor of prognosis than the one-point BMI when the pre-onset weight values were reliable.

Metabolic abnormalities in ALS are unique. Four aspects have been examined, and they seem to interact with each other: malnutrition with hypermetabolism (1,3,6), glucose intolerance (7), dyslipidemia (8), and sympathetic hyperactivity (9,10). The hypermetabolism is probably connected with the main pathomechanism in ALS, and mitochondrial dysfunction might be causative (5). Spontaneous motor neuron firing or fasciculation would augment glucose consumption in surviving muscle fibers. Skeletal muscle loss would increase insulin resistance, resulting in glucose intolerance and insulin hypersecretion (7,11). Sympathetic tone is often augmented by a central neural mechanism (9,10), which would induce hypermetabolism. Additionally, increased insulin secretion would also raise sympathetic tone and metabolic demand. All of these abnormalities, by interacting with each other, might induce progressive weight reduction and clinical deterioration.

Considering these metabolic abnormalities, physicians should pay special attention to nutritional management. We should not miss the appropriate timing of gastrostomy and nutritional therapy. Gastrostomy should be introduced for sufficient calorie intake as early as possible when patients exhibit malnutrition (12). Faster BMI reduction may be able to predict the efficacy of early gastrostomy. In addition, high-calorie nutritional therapy would be expected to prolong survival in patients with high BMI-RR (13). It is important to attempt to stop weight reduction. Clinicians should administer as many calories as possible from the early stage in patients with malnutrition. The precise amounts of necessary calories at each stage should be established in the future.

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CASE REPORT

Prenatal diagnosis of osteogenesis imperfecta type II by three-dimensional computed tomography: The current state of fetal computed tomography

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ABSTRACT **We report a case of osteogenesis imperfecta (OI) (OMIM166210) type II, in which a prenatal diagnosis was made by three-dimensional computed tomography (3D-CT). Subsequent molecular analysis revealed a recurrent, heterozygous mutation in *COL1A2*. Fetal CT is a powerful tool for visualizing the fetal skeleton and can provide a definitive diagnosis of fetal skeletal dysplasias; however, whether or not its employment for prenatal diagnosis is warranted in terms of fetal radiation risks remains controversial, both medically and ethically. Based on our experience, we review the current state of fetal CT for the diagnosis of skeletal dysplasias, with a discussion of the relevant literature.**

Key Words: genetic counseling, osteogenesis imperfecta type II, prenatal diagnosis, skeletal dysplasia, three-dimensional computed tomography

INTRODUCTION

Recent advances in fetal two-dimensional ultrasound (2D-US) have greatly facilitated the diagnosis of fetal anomalies, including fetal skeletal dysplasias. Fetal US allows visualization of cardinal manifestations of skeletal dysplasias, such as limb shortening, limb deformities, spinal maldevelopment and thoracic hypoplasia. However, these changes must be understood comprehensively to recognize the overall pattern of each type of skeletal dysplasia. This comprehensive assessment, based on specific criteria, is not an easy task. Instead, fetal three-dimensional computed tomography (3D-CT) readily demonstrates skeletal abnormalities identical to those on postnatal radiography, and has gained popularity as a modality of choice for prenatal diagnosis of fetal skeletal dysplasias; yet, whether or not fetal CT is warranted in terms of fetal radiation risks remains controversial. There are also ethical concerns. We report herein our experience with fetal CT yielding a definitive diagnosis of lethal osteogenesis imperfecta at 21 weeks of gestation, and discuss the current state of fetal CT for prenatal diagnosis of skeletal dysplasias. The relevant literature is also reviewed.

CASE REPORT

A 32-year-old Japanese woman, a primigravida, was referred for prenatal diagnosis and genetic counseling because of severe shortening of the limbs had been recognized in her fetus on US at 19 gestational weeks. The woman and her husband were healthy and their family histories were unremarkable. Repeat US showed bowing of the long bones and a narrow thorax, and a biparietal diameter of 44 mm (−2.4SD), abdominal circumference (AC) of 136 mm (−2.1SD), femoral length (FL) of 16 mm (−6.3SD), humeral length of 17 mm, radial length of 12 mm, and ulnar length of 14 mm (Fig. 1). The FL/AC ratio of 0.14, along with other findings, raised suspicion of a lethal type of skeletal dysplasia, and the parents were informed of this possibility. They requested more detailed examinations to confirm the diagnosis. Thus, fetal CT was offered as a further diagnostic option. They had meticulous genetic counseling from clinical geneticists (a pediatrician and an obstetrician), a genetic counselor, and a clinical psychologist. They were thoroughly explained about the possibility of fetal morbidity, the risks of radiation, the medical assistance in perinatal management and pregnancy continuation. As fetal CT has been warranted as a common clinical practice, its performance was not referred to the ethical committee in our institution. After the counseling, 3D-CT was performed with informed consent. CT images were obtained using a 64-slice scanner (Aquilion 64; Toshiba Medical Systems, Japan). The fetal radiation dose was estimated to be 48.4 mGy (CTDI Computed tomography dose index). The 3D-CT images showed defective calvarial ossification, a small thorax with multiple rib fractures, and bowed, and/or crumpled long bones, indicating a diagnosis of lethal OI (OI type II) (Fig. 2). Given the imaging findings, the couple opted for an elective abortion, which was performed at 21 gestational weeks. The fetus had extremely short and curved limbs. After genetic counseling, a molecular genetic analysis of DNA obtained from fetal skin fibroblasts was performed and revealed p.G433E in the *COL1A2* gene. Neither the woman nor her husband had this mutation.

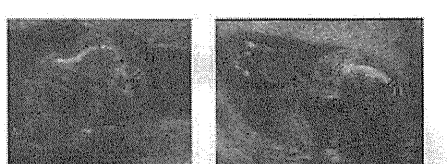

Fig. 1 Two-dimensional ultrasonography of the fetal femur (a) and humerus (b). The bones are short and curved.

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Table 1 Results of previous reports on fetal computed tomography for skeletal dysplasias

Case	Diagnosis GW	Prenatal diagnosis	Post-diagnosis
Present case	21	OI type II	OI type II
Wada et al. 2011	28	Moderate shortening of the limbs, mild narrow thorax, polyhydramnios	Kniest dysplasia
Cassart et al. 2007	33	IUGR/ACH	IUGR
	26	IUGR/ACH	SED
	36	Suspicion of ACH	ACH
	31	Asphyxiating thoracic dysplasia	SED
	30	TD	TD
	33	Suspected L3 vertebral anomaly	Isolated vertebral anomaly, hemivertebra in L3
	32	?	Isolated vertebral anomaly, fused hemivertebra in D12
	30	Isolated vertebral segmentation anomaly	Multiple vertebral segmentation anomalies
	26	Apert syndrome?	Pfeiffer syndrome
	30	Crouzon syndrome? Osteopetrosis not excluded	Osteopetrosis not excluded
	36	OI?	OI not excluded
Ulla et al. 2011	22	OI type III	OI type III
	19	OI type II	OI type IIB
	20	CD	No diagnosis
	22	OI type II	OI type II
	23	CDP	CDP
	22	TD type I	TD
Yamada et al. 2010	33	SRPs type 3	SRPs type 3
Tsutsumi et al. 2008	27	TD	TD
Miyazaki et al. 2007	31	CDP	CDP
Bonnefoy et al. 2006	38	Hypochondroplasia	Hypochondroplasia
Ruano et al. 2004	32	ACH	ACH
	27	ACH	ACH
	31	ACH	ACH
	33	OI	OI
	36	OI	OI
	32	CDP	CDP
Otera et al. 2009	33	IUGR/short femur	PFFD
Sonigo-Cohen et al. 2003	32	Bony abnormalities	Diastematomyelia

ACH, achondroplasia; CD, campomelic dysplasia; CDP, chondrodysplasia punctata; GW, gestational week; IUGR, intrauterine growth retardation; OI, osteogenesis imperfecta; PFFD, proximal focal femoral deficiency; SED, spondyloepiphyseal dysplasia; SRPs, short-rib polydactyly syndrome; TD, thanatophoric dysplasia.

DISCUSSION

Lethal OI can easily be diagnosed by clinical and radiological findings (OMIM Review OSTEOGENESIS IMPERFECTA, TYPE IIA). Most OI cases have a de novo, heterozygous mutation in one of the type I collagen genes (*COL1A1 and COL1A2*) (Niyibizi et al. 2004; Morello and Rauch 2010). However, several genes responsible for autosomal recessive OI have recently been discovered. Therefore, molecular analyses for type I collagen genes and other newly identified genes is essential for definitive genetic counseling. In addition, the possibility of parental germinal mosaicism should be taken into account. In the present case, we were able to confirm that the disease phenotype was related to a *COL1A2* mutation. This is a recurrent type of mutation previously reported in lethal OI and was likely to have been a de novo mutation in the present case (Rose et al. 1993; Marini et al. 2007).

As exemplified by the present case, there is a general consensus that skeletal imaging using fetal 3D-CT is comparable to that of conventional radiography. Thus, it is apparent that this modality is a powerful tool for making a definitive prenatal diagnosis of fetal

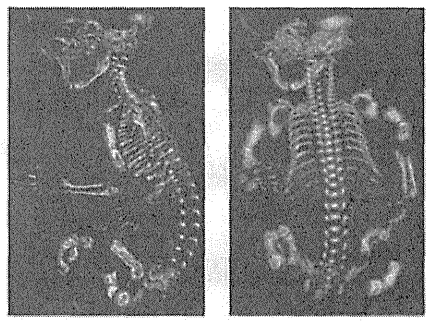

Fig. 2 Three-dimensional computed tomography images show poorly mineralized craniofacial bones, curved limbs and short thick long bones with multiple fractures.

skeletal dysplasias. The results of previous reports on fetal CT for skeletal dysplasias and fetal skeletal disorders are summarized in Table 1. Several reports deserve particular attention (Bonnefoy et al. 2006; Miyazaki et al. 2007; Tsutsumi et al. 2008; Yamada et al. 2010). Ruano et al. (2004), Cassart et al. (2007), and Ulla et al. (2011) described diagnostic capabilities of 3D-CT in fetal skeletal dysplasias (Ruano et al. 2004; Cassart et al. 2007; Ulla et al. 2011). Ruano et al. reported that 2D-US provided a correct diagnosis in four of six cases, while 3D-CT yielded the correct diagnosis in all six cases (Ruano et al. 2004). Cassart et al. were able to make a correct diagnosis in two of 11 cases using 2D-US, but in eight of the 11 cases with 3D-CT (Cassart et al. 2007). Ulla et al. reported that 3D-CT contributed to making a precise diagnosis in five of six cases with skeletal dysplasias (Ulla et al. 2011). Other reports on prenatal CT diagnosis addressed congenital diaphragmatic hernia (Urban et al. 1999), trisomy 18 and cystichygroma (Sohda et al. 1997), and agnathia-holoprosencephaly (Ebina et al. 2001).

Despite the superb diagnostic capabilities of fetal CT for the skeleton, fetal radiation risks discourage its application. The ICRP (International Commission on Radiological Protection 2000) reported that radiation risks are most significant during organogenesis and that in the early fetal period the malformation threshold is 100–200 mGy or higher. Radiation doses utilized in previous reports were less than those that raise concern by the ICRP, but were variable among the study groups. The most recent works have reported that clinically acceptable images are currently available at less than 10 mGy (Miyazaki et al., pers. comm. 2007). The 48.4-mGy X-ray dose employed in the present case was thus considered to be well below the safety threshold (ICRP Pregnancy and Medical Radiation 2000), but it was relatively higher than the most recent work. It would be necessary to reduce fetal CT radiation doses in the next study. The potential alternative to fetal CT is 3D-US, which is anticipated to provide precise diagnostic imaging for fetal skeletal dysplasias in the near future. Ruano et al. described 3D-US as providing more details of skeletal abnormalities than 2D-US (Ruano et al. 2004). Advancements in 3D-US are expected to increase the diagnostic accuracy of this technique. Unfortunately, we were not able to have an occasion to evaluate the present case by using 3D-US.

Finally, a number of clinicians and genetic counselors have questioned whether or not definitive diagnosis of fetal skeletal dysplasias is necessary for optimal genetic and obstetric management of fetal skeletal dysplasias. Meticulous US examinations have been advocated as being sufficient to provide optimal medical services. The emotional and ethical considerations of this complicated issue are beyond the scope of the present report. Further discussions of the ethical issues along with technical investigations are required.

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Original article

Enhanced expression of myogenic differentiation factors and skeletal muscle proteins in human amnion-derived cells via the forced expression of *MYOD1*

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Abstract

Objectives: Mesenchymal stem cells are expected to be an ideal cell source for cellular and gene therapy. We previously showed that cells derived from the human placenta can be induced to differentiate into myotubes *in vitro* and to express dystrophin in mdx/scid mice *in vivo*. In this study, we examined whether amnion-derived cells can be efficiently transduced and differentiated using lentiviral vectors carrying human *MYOD1*. *Methods:* The amnion-derived cells were isolated from human preterm placentas. They were transduced with the *MYOD1* vector, and mRNA levels for *MYOD1*, *MYF5*, *MYOG*, *MYH2* and *DMD* were determined by quantitative-reverse transcriptase-polymerase chain reaction, and also examined immunocytochemically. *Results:* Approximately 70% of amnion-derived cells were efficiently transduced by the lentiviral vectors. *MYOD1* activates *MYF5* and *MYOG*, *MYH2* and *DMD* after a 7-day culture. The concerted upregulations of these myogenic regulatory factors enhanced *MYH2* and *DMD* expressions. *PAX7* was below the detectable level. Both myosin heavy chain and dystrophin were demonstrated by immunocytochemistry. *Conclusions:* *MYOD1* activates *MYF5* and *MYOG*, the transcription factor genes essential for myogenic differentiation, and the concerted upregulation of these myogenic regulatory factors enhanced *MYH2* and *DMD* expressions. The amniotic membrane is an immune-privileged tissue, making *MYOD1*-transduced amnion-derived cells an ideal cell source for cellular and gene therapy for muscle disorders. This is the first report showing that amnion-derived cells can be modified by exogenous genes using lentiviral vectors. Furthermore, *MYOD1*-transduced amnion-derived cells are capable of the dystrophin expression necessary for myogenic differentiation.

Keywords: Duchenne muscular dystrophy; Mesenchymal stem cell; Cellular therapy; Gene therapy; Dystrophin; Placenta

1. Introduction

Duchenne muscular dystrophy (OMIM# 310200) is an X-linked recessive inherited disorder that affects 1 in 3500 males. The onset of Duchenne muscular dystrophyis usually before the age of 3 years, and patients die of respiratory failure around the age of 20 [1]. Duchenne muscular dystrophyis caused by structural mutations in the dystrophin gene (*DMD*), which encodes dystrophin, a large membrane-associated protein that plays an important role in linking intracellular cytoskeletal actin filaments to the sarcolemmal membrane [2]. Approximately 60% of *DMD* mutations are large deletions or insertions, whereas 40% are nonsense, missense, or small insertion-deletion mutations.

No curative therapeutic approaches for Duchenne muscular dystrophy currently exist. However, cell-based treatments in addition to gene therapy [3], exon skipping therapy [4], and read-through therapy with aminoglycosides [5] remain promising options.

Mesenchymal stem cells (MSC) are expected to be an ideal cell source for cellular and gene therapy because they can easily be obtained from bone marrow, adipose tissues, and the placenta, they are abundant and non-tumorigenic, and they have the useful characteristics of homing and chemokine secretion. MSC are already utilized for the treatment of graft versus host disease [6] and inflammatory bowel disease [7]. Several laboratories have shown that MSC can be obtained from amnion-derived cells and induced to differentiate into myocytes [8].

Although the myogenic differentiation of MSC can be induced by treating them with demethylating agents such as 5-azacythidine (5AZA), there is no marked enhancement of either *MYOD1*, the human myogenic differentiation factor 1 gene, or *MYH2* expression, nor does5AZA treatment substantially increase the myogenic differentiation of MSC [9]. In addition, there have been several attempts to enhance the myogenesis by introducing *MYOD1* into cells [10]. It was recently shown that human adipose-derived cells displayed enhanced myogenic differentiation after being forced to express *MYOD1* [11], and another group showed that forced expression of *MYOD1* led to the trans-differentiation of human fibroblasts into myotubes [12].

In this study, we introduced human *MYOD1* into amnion-derived cells using a lentiviral vector and examined the precise gene expression levels of *MYF5*, *MYOG*, *MYH2* and *DMD*. We demonstrated significant upregulations of the genes for essential transcription factors involved in myogenesis. The potential applications of*MYOD1*-transduced amnion-derived cells are also discussed.

2. Materials and methods

2.1. Isolation of human amnion-derived cells

Ethics approval for the tissue collection was granted by the Institutional Review Board of Tokyo Women's Medical University, Japan. Written informed consent was obtained prior to sample collection. Amnion tissue samples were obtained from normal full-term pregnancies at the time of caesarean section before the onset of labor. None of these pregnancies were complicated by premature membrane rupture or chorioamnionitis. The placentas were processed within 24 h of collection; i.e., they were thoroughly washed with phosphate-buffered saline (a solution containing sodium chloride, sodium phosphate, potassium chloride and potassium phosphate), and, after separation from the placentas, the amnions were minced into 5 mm sections using knives on a clean bench. The amnion tissue was placed in collagen I coated dishes (Iwaki, Japan), and after 20 min, Mesenchymal Stem Cell Basal Medium (MSCBM, Lonza, USA) was carefully poured onto the attached cells, which were then maintained at 37 °C in 5% CO_2. After 48 h, the non-adherent cells were removed, and the medium was changed twice a week. After about one week, a few colonies were found in the dishes. At 70–80% confluence, the amnion-derived cells were harvested with 0.5% Trypsin–EDTA (Life Sciences, USA) and plated onto new dishes. Cells were processed from 24 placentas, and primary cultures from 8 placentas were used for this study.

2.2. Flowcytometric analysis

The amnion-derived cells were used for fluorescent activated cell sorting (FACS) analysis employing the EPICS ALTRA XL-MCL analyzer (Beckman Coulter, USA), and the data were analyzed with EXPO™32 ADC software (Beckman Coulter). Antibodies against human CD14, CD29, CD34, CD44, CD45, CD73, CD105, CD166, HLA-ABC, and HLA-DR were obtained from Beckman Coulter and BD Biosciences Pharmingen (USA), AbD Serotec (UK) and Cytognos (Spain).

2.3. Production of lentiviral vectors and MYOD1 transduction of human amnion-derived cells

A full-length human *MYOD1* cDNA clone (Genome Network Project Clone, WW01A62C23) was provided by the RIKEN Bioresource Center (Ibaraki, Japan) through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan [13–16]. A lentiviral vector carrying the *MYOD1* cDNA, pLenti6/human*MYOD1*, was constructed using the pLenti6/UbC/V5-DEST Gateway Vector kit and the ViraPower Lentiviral Expression System (Life Technologies, USA). A GFP expression vector, pRRL.PPT.SF.IRES-GFP, was kindly provided by Taiju Utsugisawa.

Three micrograms of the purified pLenti6/UbC/V5-DEST – human*MYOD1* cDNA and pRRL.PPT.SF.IRES-GFP were used for the transfection of 4×10^6 293FT cells together with Lipofectamine 2000 (provided with the kit) reagent and ViraPower packaging Mix (provided with the kit). After 48 h, the supernatant

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was collected. Eight milliliters of viral supernatant were added to 6–8 × 10^5 amnion-derived cells. To examine the transfection efficiency of our procedure, the GFP expression of the cells was analyzed by FACS.

2.4. In vitro myogenesis

The amnion-derived cells were transduced with the pLenti6/UbC/V5-DEST – human *MYOD1* supernatant and seeded onto collagen I coated cell culture dishes (IWAKI) at a density of 1 × 10^4 per ml in growth medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% fetal bovine serum) for 24 h. The cell cultures were then washed twice with phosphate-buffered saline (–), and maintained in differentiation medium (DMEM supplemented with 2% horse serum (Iwaki, Japan)). The medium was changed twice a week until completion of the experiment.

2.5. Quantitative-reverse transcriptase-polymerase chain reaction using MYOD1-transduced amnion-derived cells

Total RNA was purified from the *MYOD1*-transduced amnion-derived cells using the RNeasy mini kit (QIAGEN, Germany) at 7 and 14 days after transduction. Two micrograms of total RNA were subjected to reverse-transcription using Expand Reverse Transcriptase (Roche, USA). The gene expression levels of *MYOD1*, *MYF5*, *PAX7*, *MYOG*, *MYH2*, and *DMD* were analyzed using the quantitative-reverse transcriptase-polymerase chain reaction (Q-RT-PCR), the primers listed in Supplementary Table 1, and Mx3000™ (Stratagene, USA). The non-transduced amnion-derived cells were designated the day 0 cells. Q-RT-PCR was performed at 95 °C for 10 min for 45 cycles, with each cycle consisting of 95 °C for 15 s, followed by treatment at 60 °C for 60seconds after completion of the last cycle. Relative gene expression levels were calculated using RNA extracted from normal human skeletal muscle myoblast cells (Lonza), which had been cultured for 4–5 days using the SkGM-2 BulletKit (Lonza) or for 7 days in total DMEM supplemented with 2% horse serum as a myoblast control [17].

2.6. Immunocytochemical analysis

The cultures were fixed in 4% paraformaldehyde and stained with either a mouse anti-human dystrophin IgG1 monoclonal antibody (NCL-DYS2, Novocastra, UK) or a mouse anti-myosin heavy chain IgG2 monoclonal antibody (MF-20, Developmental Studies Hybridoma Bank, USA). The cells were visualized with appropriate AlexaFluor488 goat anti-mouse IgG secondary antibodies (Invitrogen, USA). Total cell nuclei were stained with Hoechst solution (Sigma Aldrich, UK).

3. Results

3.1. Morphology of the amnion-derived cells

We isolated amnion-derived cells from placentas, and a large number of primary cells were successfully obtained. These cells consisted of small fibroblast-like and cobblestone-like cells (Fig. 1a), and after 3 passages, they had formed a homogeneous population of the fibroblast-like cells (Fig. 1b). The yield was approximately 2 × 10^7 cells per gram of amnion tissue after three weeks.

3.2. Surface markers of amnion-derived cells

The surface marker expressions of amnion-derived cells were evaluated by flowcytometric analysis (Fig. 2). After 3 passages, the amnion-derived cells were positive for CD29, CD44, CD73, CD105, CD166, and HLA-ABC. However, they did not express any hematopoietic lineage markers such as CD34, CD14, CD45 or HLA-DR.

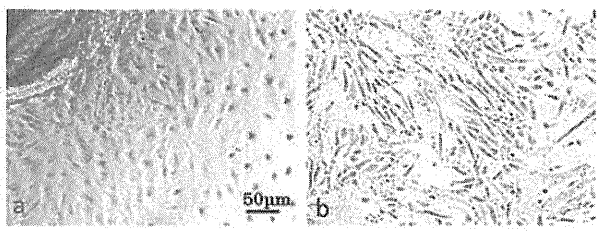

Fig. 1. Photomicrographs of amnion-derived cells in primary cultures in which two cell types, cobblestone- and fibroblast-like cells, can be seen (a). After three passages, the cells had formed a homogeneous population of fibroblast-like cells (b). Scale bar: 50 μm.

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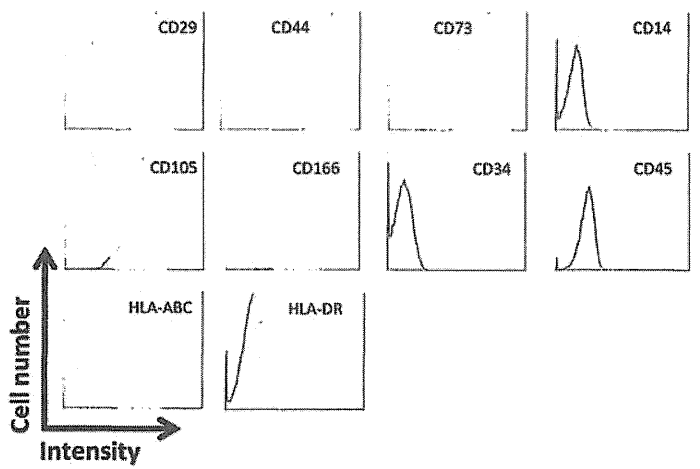

Fig. 2. Surface marker expressions of amnion-derived cells were evaluated by flowcytometric analysis after 3 passages. They expressed the following MSC markers: CD29, CD44, CD73, CD105 CD166, and HLA-ABC. However, they did not express any hematopoietic lineage markers such as CD34, CD14, CD45, or HLA-DR.

3.3. Transduction of amnion-derived cells with a GFP vector

The amnion-derived cells were transduced with a GFP vector, and the transduction efficiency of our procedure was evaluated by flowcytometric analysis after 72 h. In each experiment, approximately 70–80% of amnion-derived cells were positive for GFP (data not shown).

3.4. Q-RT-PCR of MYOD1 transduced amnion-derived cells

The mRNA levels of *MYOD1*, *MYF5*, *MYOG*, *PAX7*, *MYH2*, *and DMD* in the *MYOD1*-transduced amnion-derived cells were determined by Q-RT-PCR. Forced expression of human *MYOD1* in amnion-derived cells markedly enhanced their *MYF5* and *MYOG* gene expression levels after 7 days of culture (Fig. 3). On day 7, the *MYOD1*mRNA level of these cells was increased 50-fold as compared to that observed on day 0. The mRNA level corresponded to 4.8% of that in the control myoblasts. On day 14, the *MYOD1*mRNA level had decreased to 1.1% of that in the controls, suggesting that the enhanced *MYOD1* mRNA expression was transient.

It should be noted that *MYF5*gene expression was highly upregulated in the *MYOD1*-transduced amnion-derived cells. On day 7, it was increased by over 500-fold, and the relative mRNA level had reached 5.6% of that in control myoblasts. However, on day 14, the *MYF5* expression of these cells had been almost completely abrogated.

The *MYOG* mRNA level in the amnion-derived cells was extremely low (0.02%) on day 0. On day 7, it had reached 0.125% of that in the control myoblasts, but was undetectable by day14.

On days 7 and 14 the mRNA levels of *MYH2* were 0.11% and 0.33% and those of *DMD* were 20% and 28%, respectively, of corresponding levels in the control cells. These results suggest that genes encoding skeletal muscle proteins were activated following the concerted activation of myogenic regulatory factors such as *MYOD1*, *MYF5*, and *MYOG*. On the other hand, the level of *PAX7* was not measurable in either the non-transduced or transduced amnion-derived cells (data not shown).

3.5. Immunocytochemistry of myogenic differentiated cells

The MYOD1-transduced amnion-derived cells were subjected to immunocytochemical analysis after a 28-day culture in differentiation medium. Both myosin heavy chain 2 and dystrophin were immunologically detected in their cytosol and nuclei, suggesting these cells to be capable of differentiating into myotubes (Fig. 4).

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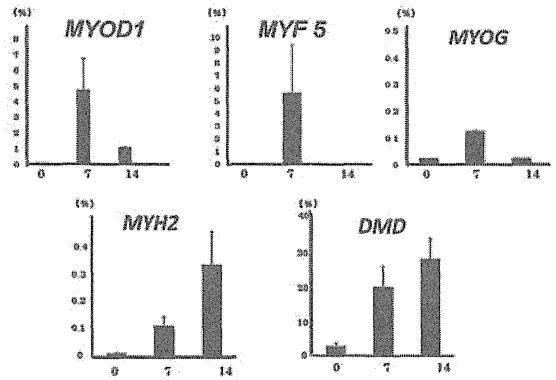

Fig. 3. The mRNA levels of *MYOD1*, *MYF5*, *MYOG*, *PAX7*, *MYH2*, and *DMD* in the *MYOD1* transduced amnion-derived cells were determined by Q-RT-PCR using the Universal Probe Library system (Roche). Relative gene expression levels were calculated using normal human skeletal muscle myoblast cells (Lonza) as a control. Day 0 represents the mRNA level of the amnion-derived cells on the day of transduction. Days 7 and 14 represent the mRNA levels of the *MYOD1*-transduced amnion-derived cells. The mRNA levels of the cells were as follows: *MYOD1*, 0.10 ± 0.05%, 4.8 ± 2.0%, and 1.09 ± 0.07%; *MYF5*, 0.013 ± 0.001%, 5.6 ± 3.8%, and < 0.0025%; *MYOG*, ≦0.00%, 0.13 ± 0.01%, and <0.0025%; MYH2, 0.01 ± 0.001%, 0.11 ± 0.03%, and 0.33 ± 0.12%; and *DMD*, 2.8 ± 1.0%, 20 ± 6.0%, and 28 ± 6.0%. The mRNA levels of the *MYOD1*-transduced amnion-derived cells were markedly upregulated, as shown in the figure; however, *PAX7* was not detected in either the untreated or the transduced amnion-derived cells.

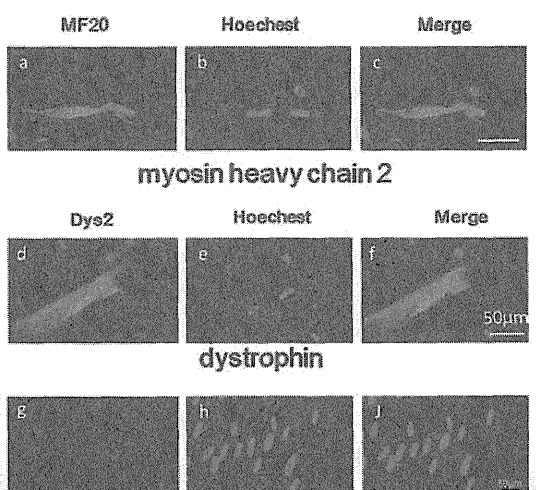

Fig. 4. Expressions of muscle specific genes during the differentiation of amnion-derived cells. *MYOD1*-transduced amnion-derived cells were cultured in differentiation medium for 28 days and stained using immunohistocytochemical methods. (a) Myosin heavy chain molecule (green, MF20); (b) nuclei (blue, Hoechst); (c) merged image of a and b; (d) dystrophin molecule (green, Dys2); (e) nuclei (blue, Hoechst); (f), merged image of d and e;The untreated amnion-derived cells expressed neither myosin heavy chain (g–h) nor dystrophin (data not shown). Scale bars: 50 μm.

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4. Discussion

Myogenesis is classified into two modes, skeletal myogenic differentiation during development and regeneration mediated through satellite cells after injury. Previous studies have revealed that several transcription factors are essential for regulating embryonic and adult muscle formation. Among these factors, MyoD, Myf5, and myogenin are considered to be master transcription factors that are essential for myogenesis and are known as myogenic regulatory factors [18]. During myogenesis, *MYOD1* and *MYF5* are able to functionally substitute for one another [19]. In contrast, MyoD and Myf5 have distinct functions in the processes involved in recovery from muscle injury: MyoD is indispensable for the commitment of satellite cells to become myogenic precursor cells, and Myf5 plays an important role in myoblast proliferation [20]. On the other hand, myogenin is important for terminal muscle differentiation and lineage maintenance [21]. In this study, we clearly showed *MYOD1* transduction to activate both *MYF5* and *MYOG*, leading to myogenic differentiation resembling the developmental stages between myogenic progenitors and myoblasts. It is still unclear whether the concerted activation of *MYF5* and *MYOG* is due to direct transactivation by MyoD. Nonetheless, sequential activation of myogenic differentiation factors subsequently increased the gene expression levels of *MYH2* and *DMD*. As a result, the myosin heavy chain2 and dystrophin were immunocytochemically detected, as shown in Fig. 4. However, we were unable to demonstrate the protein expression of MYOD1, MYF5, MYOG, or PAX7, since examining the expressions of transcription factors by Western blotting or other immunological procedures is quite difficult.

Satellite cells, which are generated around myofibers during fetal development, express another transcription factor, Pax7. They are mitotically quiescent, but are activated in response to the stress induced by muscle injury [22]. Although the *MYOD1*-transduced amnion-derived cells did not display *PAX7* expression, the biological significance of this transcription factor remains obscure.

We previously demonstrated that human MSC transplanted into skeletal muscles of mdx mice successfully differentiated and fused with murine muscles, suggesting cellular therapy to be a promising strategy for Duchenne muscular dystrophy [23]. A previous report showed that dystrophin Dp71 and dystrophin-associated proteins are co-localized to the nuclei of muscle cells. This result implies that allogenic *MYOD1*-transduced amnion-derived cells would be a useful tool for cellular therapy [24].

The amniotic membrane is an immuno-privileged tissue and has been used as a biological membrane for treating burns, injuries, and skin ulcers as well as for corneal transplantation [25–27], and the transplantation of amniotic membrane-derived cells has been experimentally applied to the treatment of lung fibrosis [28]. Recently, a preclinical study reported their use for regenerative therapy targeting central nervous system tissues [29]. It is also known that the amniotic epithelium produces anti-inflammatory and growth factors that are beneficial for the treatment of inflammatory corneal diseases [30]. Taken together, these observations explain the increasing attention that the human amniotic membrane has received due to its anti-scarring, anti-inflammatory, and wound-healing properties, as well as its multipotent differentiation ability and immunomodulatory features [31].

In conclusion, the placenta has several advantages as the cellular source for devising novel cellular and gene therapies, especially for muscle disorders. The placenta can be obtained non-invasively, the amniotic membrane has been shown to be an immune-privileged tissue and*MYOD1*-transduced amnion-derived cells are capable of the dystrophin expression needed for myogenic differentiation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.braindev.2012.05.012.

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Exoskeleton Robot Control based on Cane and Body Joint Synergies

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Abstract— Several methods have been investigated and realized for operation of exoskeleton robots for assistance of human gait. These systems perform motion intention estimation using the bioelectrical signals of muscle activation, body gestures and kinesiological information, or a mixed combination in a hybrid system. For motion intention estimation of the lower limb(s), information of the lower limbs is usually utilized. However, human gait is not only the function of the lower limbs, but also coordination between upper and lower limbs, adding to balance and cognitive functions as well. In this study, we investigate on how to utilize the synergies of upper and lower limbs of human walking in exoskeleton robot control by using the cane (walking aid). We analyse the synergies of human gait with cane in healthy subjects by means of Principal Component Analysis (PCA) in order to investigate the usability of cane for robot-assisted motor rehabilitation. We also implement a semi autonomous control for an exoskeleton robot, single leg version of HAL (Hybrid Assistive Limb) suit, based on the cane and body joint synergies.

I. Introduction

Several methods of human intention estimation have been investigated and realized for operating of human assistive exoskeleton robots [1], [2], [3], [4], [5]. These methods vary according to the intended assistance level (ex. complete reproduction of movement, support of movement etc.), the level of injury or dysfunction of the patient (ex. muscle weakness, partial or complete paralysis etc.), and the structure of the assistive robot itself.

The biological signals are reliable information to estimate human motion intention. The hybrid control algorithm of HAL [1] consists of a human voluntary control and an autonomous control. The wearer's voluntary muscle activity is obtained from the bioelectrical signals, detected at the surface of the muscles, and then the required assist torque of the actuators is computed from the estimated joint torque. An autonomous control is also implemented based on the predetermined motion primitives, together with the voluntary control method.

However, in the case of neuronal injury/dysfunction such as stroke related hemiplegia or spinal cord injury, biological signals are different from that of healthy subjects or even not available. Therefore, reference trajectory for the assisted limb(s) needs to be computed, and the motion intention is required to be estimated in different ways[2], [6]. Kawamoto et al. [6] developed a control system for single leg version of HAL [7] by using FRF (Floor Reaction Force) sensors to detect the gait phase shifting intended by the patient. The robot is then operated by assembling segments of reference trajectories to restitute the motion of the impaired limb. And more extended work has been realized for paraplegia patients in [2] with the use of body posture information to convey the motion intention of paraplegic patients. For another rehabilitation robot, LOPES, Vallery et al. [8] generated the reference trajectory for the impaired (hemiplegic) limb by utilizing the inter-joint coupling of DoFs in the lower limbs of healthy subjects, and the motion of the healthy limb. By this method they aimed to keep the coordination of the healthy and assisted limbs, and to insure coherency of healthy and assisted limb motion.

In these paradigms, motion information from the lower limb(s) is used for estimation. However, human gait is not only the function of lower limbs, but also a coordination between upper and lower limbs [9], [10], [11], [12], [13]. Ferris et al. [14] suggested using the arms swing to facilitate lower limb muscle activation because of the neuronal coupling between upper and lower limbs in rhythmic locomotion tasks. Stephenson et al. [15] have pointed that high functioning stroke patients preserve the ability to coordinate the motion of upper and lower limbs, and also suggested that the use of sliding handles in gait rehabilitation could be useful. Behrman and Harkema [16] have also shown that reciprocating arm swing in a natural and coordinated form facilitates stepping. The field of robot assisted rehabilitation using exoskeleton robots haven't yet explored the possibilities to incorporate the upper limbs in the control strategy. In this research we aim to investigate on how to utilize the upper and lower limbs synergies of human walking in exoskeleton robot control. We consider the rehabilitation of hemiplegia where the patient have a healthy side and an affected side, and we try to formulate a control system that incorporate the motion of the unaffected arm to provide assistance for the affected leg. Hence we aim not only to encourage the arm swing motion, but also to utilize it in the process of motion intention estimation as a "voluntary" input to the system.

Vallery et al. [8], [3] suggested a method to estimate the motion of impaired limb(s) from the motion of other healthy limb(s) depending on the interjoint synergies extracted from healthy people. This approach can be used to estimate the motion trajectory of the affected (in the case of hemiplegia) from the motion of the arms and the healthy leg. But there are still important events not being detected in this case, which

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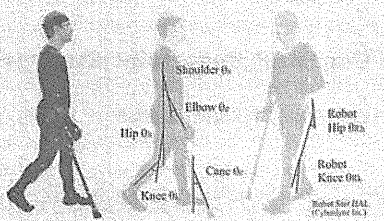

Fig. 1. Measured angles for gait with cane analysis and system implementation.

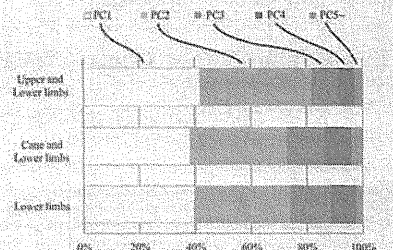

Fig. 2. Group mean ratios of the first 4 principal components to the overall data for each of the three sets of variables.

are the gait phases of the affected leg. The initial contact and Toe-off are especially important since they define the Stance and Swing conditions, which require different control demands from robotics perspective.

Hemiplegic patients use a cane in the healthy arm (contralateral to the affected leg) to support their weight and balance [17], [18], and the cane's motion is in phase with the affected leg. Having a cane, the arm is more significantly incorporated in the coordination of gait, and the movement of cane can be expected to be naturally a part the above mentioned synergies. Therefore, we propose to use the cane to capture the arm's motion and the ground contact information. For this purpose, we analyze healthy patterns of walking with/without cane by means of Principal Component Analysis (PCA) in order to measure the cane-limb coupling, and the inter-subject similarity of locomotion pattern using the cane. We perform motion intention estimation by using the motion data of one leg together with the movement of the cane to generate the reference trajectory for the contralateral leg. And finally, we examine the proposed method with the single leg version of robot suit HAL [7].

II. Analysis of Joint Synergies

In order to investigate the cane movement and the interjoint coupling with the cane during locomotion, we conducted gait analysis for walking with cane. We recorded gait patterns of seven healthy subjects for walking with/without a cane. The joint angles and angular velocities of the shoulder, elbow, hip and knee joints for the right and left side limbs, as well as the tilting angle and angular velocity of the cane were computed (Fig.1). Three cases were inspected with: (i) Joint coupling of the lower limbs. (ii) Joint coupling of the upper and lower limbs. (iii) Coupling of the cane and the lower limbs.

PCA as a method of calculating the optimal linear combinations of a set of variables [19] has been used frequently to approximate the inter-joint couplings, synergies, by linear combinations of Degrees of Freedom [20]. We therefore use PCA in this study to inspect the synergies (represented by principal components) for different sets of variables in the walking trials. We also evaluate the inter-subject similarity in using the cane through a small set of variables.

A. Experimental Setup

a) Data recording: Full body kinematics were recorded at 120Hz with a 3D optical motion capture system (MotionAnalays Inc.). Infrared reflective markers were fixed on 42 anatomical landmarks on the subject's body, and four markers were fixed on the cane to extract it's movement. For the measurement of the cane's contact with ground, three FSR (Force Sensitive Resistor) sensors were fixed on the bottom of the cane to detect its initial contact, full contact, and leaving from the ground. The data from the FSR sensors were transferred to the motion capture system through wireless communication, and synchronized with the motion capture data.

b) Subjects: Seven subjects participated in this experiment. They are males, healthy, without physiological issues of limb coordination. Experiments were done with a written informed consent. Subjects performed two trials of walking on a treadmill each for 20 seconds. The trials were performed at 2 km/h with and without the cane. Subjects were just briefly introduced to the goal of the experiment. All the subjects were right handed, and they all used the cane in the right hand.

c) Data processing: Marker labeling and model linkages were created offline. All marker data was then filtered with a two-pass, 4th order, zero phase shift, 6-Hz cut-off frequency butterworth filter. Missing marker data was interpolated with a cubic spline. Joint angles were calculated from 3D markers coordinates for the flexion and extension in the sagittal plane of the following 8 joints: leg hip, knee, arm shoulder, elbow of both right and left sides. As well as the tilting angle of the cane also in the sagittal plane. Stride cycles were identified from local maxima of the right hip angle. The angle trajectories were normalized to 0 mean and 1 standard deviation and then normalized to their average stride length to exclude step to step variations.

B. Joint-cane coordination

PCA was performed on three sets of variables. Two sets of variables are the joint angles of lower limbs with their derived angular velocities and the joint angles of upper and lower limbs with their derived velocities. The other set of

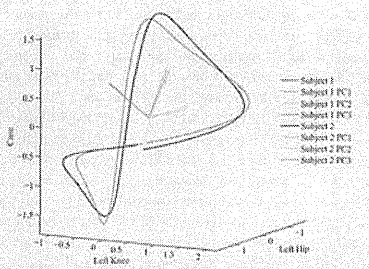

Fig. 3. Trajectories of the Left hip, Left Knee and Cane angles (normalized to 0 mean and 1 standard deviation) for two subjects with their corresponding Principal Component vectors.

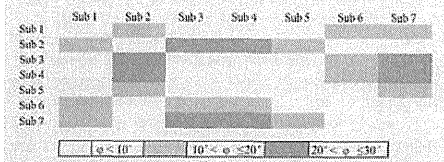

Fig. 4. Angle between the first PCs among the subjects. White, Light gray and Dark gray boxes correspond to angles less than 10°, angles between 10° and 20°, and angles between 20° and 30° respectively.

variables is the joint angles of the lower limbs and the tilting angle of the cane with their derived angular velocities. We investigated the number of Principal Components (PCs) accounting for the major part of data variation extracted from each set of variables among the subjects. The accumulated proportion of the first Principal Components (PCs) that exceeds 95% is considered in this study. The first four Principal Components accounted for more than 95% of the data for all the three sets of variables (Fig.2), except for subjects 5 and 7 while walking with cane, where the percentage of their first four Principal components was 94.48% and 94.67% respectively, which still represents a major part of the data variation. Although it is difficult to interpret the meaning of Principal Components in this case, it is observed that the cane is well incorporated into the inter-joint synergies in gait.

C. Inter-subject similarity

For evaluating the inter-subject similarity we performed PCA only on the three variables: left leg hip, left leg knee and the cane's tilting angle. We elected these variables since all subjects were right handed and used the cane in the right hand, and patients usually use the cane contralateral to the affected leg [17], [18]. The angle trajectories of the left leg and cane were also considered alone to exclude variations resulting from other variables. We then used the angles between the first principal component vectors among the subjects (illustrated in Fig.3) as a measure of similarity in using the cane [20], [21].

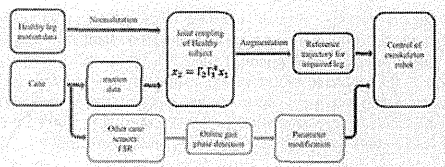

Fig. 5. Functional schematic of the suggested semi-autonomous control system.

The angle between the first PCs of each pair of subjects was less than 9.25 degrees on average. Although it was not consistent among the subjects as can be seen in Fig.4, these low values of the angles between principal components indicate a similar way of using the cane.

III. Joint Synergies based Control

From the gait analysis with cane, we found sufficient coupling between lower-limb motion and cane movement. We therefore propose to use the cane as an interface for the control of an exoskeleton robot in order to support hemiplegia patients while incorporating the arm's motion. We developed a cane equipped with motion and ground contact sensors for controlling the single leg version of HAL suit. We then propose a semi-autonomous control system that consists of three basic blocks as shown in Fig.5.

1) Reference trajectory generation for the impaired leg, using the motion data of the cane and the healthy leg, and the coupling of cane-lower limbs of healthy subjects..
2) Gait phase detection using the cane's ground reaction sensors.
3) Operating the exoskeleton robot using generated reference trajectory, and the detected gait phases.

A. Reference Trajectory Generation

To generate the reference trajectory for the intended limb one approach is to assemble it from segments of normal walk trajectories, and then to scale and synchronize the segments with the subject's intended motion. Another approach is to compute the reference trajectory continuously using the motion of other healthy limbs, and the inter-joint coupling of healthy subjects. Based on the previous analysis, we elect to use the second approach. Hence, we propose to compute the reference trajectory for the intended "hemiplegic" leg from the motion of the cane and the other "healthy" leg. In this manner, the assisted motion will be based on the motion of the healthy leg and the cane (capturing the arm motion) as well.

Implementation is done based on PCA with a method called Complementary Limb Motion Estimation (CLME) suggested in [8], [3]. In this method, the matrix of the principal components (eigenvectors of covariance matrix of the data) is rearranged to solve for some of the variables that are assumed to be unknown, from the remaining known

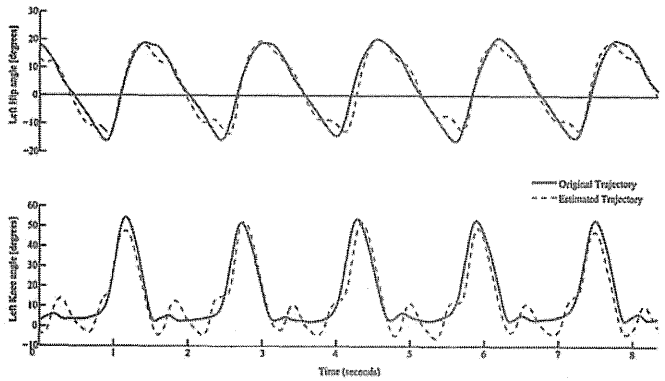

Fig. 6. Estimated angle trajectories of the Left leg hip and knee (red dashed line), and the original trajectories (black continuous line).

variables. When Γ is the matrix of the eigenvectors sorted in descending order in respect to the eigenvalues, then the original data x is mapped to the new coordinates y with the formula:

$$y = \Gamma^T x, \tag{1}$$

where the component y_1 has the maximum variance of the original data, and y_2 is the second, and so on. And since Γ is an orthonormal matrix, then x can be reconstructed from y by:

$$x = \Gamma y. \tag{2}$$

CLME methods suggests that, if $x \in \mathbb{R}^d$, and $y \in \mathbb{R}^p$, $p < d$, and there is a $d - p$ known number of x dimensions that is at least equal to the dimensionality of y. Then Γ can be separated for the known components $x_1 \in \mathbb{R}^{(d-q)}$ and the unknown components $x_2 \in \mathbb{R}^q$ as follows:

$$x_1 = \Gamma_1 y, \qquad x_2 = \Gamma_2 y, \tag{3}$$

where $\Gamma_1 \in \mathbb{R}^{(d-q)\times p}$ and $\Gamma_2 \in \mathbb{R}^{q\times p}$ are the corresponding submatrices of Γ. And therefore, the unknown x_2 can be solved from the known x_1 by:

$$x_2 = \Gamma_2 \Gamma_1^{\#} x_1, \tag{4}$$

where $\Gamma_1^{\#}$ is the left pseudoinverse of Γ_1:

$$\Gamma_1^{\#} = \left(\Gamma_1^T \Gamma_1\right)^{-1} \Gamma_1^T. \tag{5}$$

In the case of hemiplegia, the unknown variables are the joint trajectories of the affected leg, and the known variables are the joint trajectories of the cane and the healthy leg. Therefore, we consider the eigenvectors matrix extracted from walking with cane trials, and rearrange this matrix to compute the joint trajectories of the affected leg from the trajectories of the cane and the healthy leg (angles and angular velocities are both considered). In this way we add the posture of the cane as another variable to the estimation process, which is controlled directly by the arm, and thus conveying the arm's motion together with the healthy leg in the estimation process. To average the model we used the average gait cycle trajectories of all the subjects, we aligned the trajectories to the average mean for each variable among the subjects, and scaled them to the average minimum and maximum values for each variable among the subjects. We created an averaged principal components (eigenvectors) matrix by performing PCA of the concatenated trajectories of all the subjects. After that, we used it to compute the left leg trajectories by using the trajectories of the cane and the right leg (considering the experimental setup in Fig.7). Fig.6 shows the estimation result for the angle trajectories of one subject compared to the original trajectory recorded by the motion capture system.

B. Gait Phase Detection

Identifying the gait phases is important in robot assisted locomotion for detecting the intended motion, and for setting the parameters of the robot actuators according to different gait phases [2], [6]. Since we observed that the cane is incorporated in the inter-limb synergies and can be used in the estimation process, we also propose to detect the gait phases from the cane to provide the user with a simple and intuitive tool for feeding his intention to the robot. A normal gait cycle can be divided into eight gait phases: Initial Contact (IC), Loading Response (LR), Mid-Stance (MSt), Terminal Stance (TSt), PreSwing (PSw), Initial Swing (ISw), Mid-Swing (MSw) and Terminal Swing (TSw)[22]. The goal of gait phase detection is for setting of the controller's parameters of the assistive robot, in which case only detection of stance and swing conditions is important. For this purpose