らの健康教育，成人になってからの飲酒や喫煙などの日常生活習慣の改善，また疾病の早期発見・早期治療も重要であり，人生のステージごとの疾病予防を考えていかねばならない。

疾病予防のためには適度な運動，肥満防止，喫煙をしないこと，適量の飲酒，十分な休養，適切な栄養摂取が重要なことなどについては，現在まで数多くの研究が繰り返し行われ，確認されている。しかし，生活改善について何がどの程度に重要なのかは性別や年齢，体質によって異なり，個人差が大きい。疾病予防のための理想的な生活習慣は個人個人により異なっているといえる。将来，個人個人の全ゲノムが容易に解析できるようになれば，どういう疾病にかかりやすいのか，疾病の予防のために生活をどのようにしたらもっとも効果があるのかを個人個人で明らかにできるようになるだろう。疾病予防のあり方は，今後大きく変わっていく可能性がある。

文　献

1) 下方浩史：平成養生訓一百歳まで元気に生きるための知恵，世界文化社，東京，2005
2) Andres R：Effect of obesity on total mortality. Int J Obesity **4**：381-386, 1980
3) 下方浩史：理想的肥満度と長寿．治療 **80**：1426-1430，1998
4) Tchernof A, Poehlman ET：Effects of the menopause transition on body fatness and body fat distribution. Obes Res **6**：246-254, 1998
5) Ruegsegger P, Dambacher MA, Ruegsegger E：Bone loss in premenopausal and postmenopausal women. J Bone Joint Surg Am **66**：1015-1023, 1984
6) Langlois JA, Harris T, Looker AC, et al：Weight change between 50 years and old age is associated with the risk of hip fracture in white women aged 67 years and older. Arch Intern Med **156**：989-994, 1996
7) Losonczy KG, Harris TB, Cornoni-Huntley J, et al：Does weight loss from middle age to old age explain the inverse weight mortality in old age? Am J Epidemiol **141**：312-321, 1995
8) Zhu L, Viitanen M, Guo Z, et al：Blood pressure reduction, cardiovascular disease and cognitive decline in the mini-mental state examination in a community population of normal very old people：a three-year follow-up. J Clin Epidemiol **51**：385-391, 1998
9) Morgan RE, Palinkas LA, Barrett-Connor E, et al：Plasma cholesterol and depressive symptoms in older men. Lancet **341**：75-79, 1993
10) Miyaki K, Murata M, Kikuchi H, et al：Assessment of tailor-made prevention of atherosclerosis with folic acid supplementation：randomized, double-blind, placebo-controlled trials in each MTHFR C677T genotype. J Hum Genet **50**：241-248, 2005
11) Deeb SS, Fajas L, Nemoto M, et al：A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet **20**：284-287, 1998
12) Yoshida T, Sakane N, Umekawa T, et al：Mutation of beta 3-adrenergic-receptor gene and response to treatment of obesity. Lancet **346**：1433-1434, 1995
13) Sakane N, Yoshida T, Umekawa T, et al：Beta2-adrenoceptor gene polymorphism and obesity. Lancet **353**：1976, 1999
14) Kogure A, Yoshida T, Sakane N, et al：Synergic effect of polymorphisms in uncoupling protein 1 and beta3-adrenergic receptor genes on weight loss in obese Japanese. Diabetologia **41**：1399, 1998
15) Hunt SC, Geleijnse JM, Wu LL, et al：Enhanced blood pressure response to mild sodium reduction in subjects with the 235T variant of the angiotensinogen gene. Am J Hypertens **12**：460-466, 1999
16) Kitamura I, Ando F, Shimokata H, et al：Effects of the interaction between lean tissue mass and estrogen receptor α gene polymorphism on bone mineral density in middle-aged and elderly Japanese. Bone **40**：1623-1629, 2007
17) 下方浩史：長期縦断研究の目指すもの．Geriatric Medicine **36**：21-26，1998
18) Shimokata H, Ando F, Niino N：A new comprehensive study on aging—the National Institute for Longevity Sciences, Longitudinal Study of Aging(NILS-LSA). J Epidemiol **10**：S1-S9, 2000
19) 下方浩史，安藤富士子：長期縦断疫学で分かったこと．日老医誌 **45**：563-572，2008
20) 安藤富士子，西田裕紀子，下方浩史：認知機能の加齢変化とアンチエイジング．Monthly Book Medical Rehabilitation 増刊号，アンチエイジングとリハビリテーション(印刷中)

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特集：ロコモティブシンドロームと生活習慣病

2．ロコモティブシンドロームの疫学

2）運動器疾患の長期縦断疫学研究

Shimokata Hiroshi 下方 浩史　Ando Fujiko 安藤富士子

ライフ・サイエンス

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ロコモティブシンドロームと生活習慣病


下方　浩史

2．ロコモティブシンドロームの疫学

2）運動器疾患の長期縦断疫学研究

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はじめに

骨粗鬆症や関節症障害などの運動器の障害によって，要介護となる高齢者が増加している．運動器障害による要介護のリスクが高まっている状態を運動器症候群（ロコモティブシンドローム）という．高齢者における関節疾患のほとんどは変形性関節症であり，また高齢者の骨折は骨粗鬆症が主な要因となっている．吉村ら[1]によると，ロコモティブシンドロームの患者数は，変形性関節症と骨粗鬆症に限っても4,700万人（男性2,100万人，女性2,600万人）に達すると推計され，患者数は将来さらに増大していくものと思われる．また，運動器症候群による運動不足は，メタボリックシンドロームや認知症の要因となるとも考えられて，運動器症候群の予防に関する研究は，日本において今後の進展が強く望まれる分野である[2)]．

運動器疾患には縦断研究はなぜ必要か

加齢による変化を検討する方法には，大きく分けて横断的方法と縦断的方法の２つがある[2)]．縦断的研究は同一の個人を継続して観察し，加齢による実際の心身の変化，加齢に関連する要因，発育，発達，老化，寿命などをとらえようとするものである．一方，様々な年齢を含む集団を設定して，種々の検査を一度に実施し，１歳ごとのあるいは５歳，10歳ごとの年齢群で検査値がどのように異なるのかを検討し，その差を加齢変化とする方法が横断的研究である．一度の調査で終了してしまう横断研究に比べて，経時的な追跡を行う縦断研究は結論が出るまでに数年から10年以上もの期間を要し，調査を継続するための費用や人材の確保も必要である．しかし，横断的観察のみでは加齢による変化を正確にとらえることができない．

運動器症候群の予防方法を解明するためには，その危険因子を明らかにすることが必要である．無作為抽出された一般住民を対象とした長期にわたる縦断的研究は，一般住民の間での運動器疾患罹患の実態を明らかにするとともに，栄養や運動，疾病罹患，飲酒や喫煙などの生活習慣，遺伝的素因などと，加齢に関わる運動器疾患の発症との関連を解明するために不可欠である．こうした研究により，どのような素因をもち生活を送っている人が，どのような確率で運動器疾患に罹患していくのか，どのように対策を取れば，どのくらいの確率で予防できるのかを明らかにすることができる．

老化に関する長期縦断疫学研究

われわれは，1997年11月に「国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）」を開始した．１日の検査人数は７人で，毎日年間を通して詳細な老化に関連する検査を行っている[3-6)]．平成12年４月に2,267人の基礎集団が完成し，以後は２年ごとに検査を繰り返し実施し，現在は第７次調査を実施している（図１）．対象者は長寿医療研究センター周辺の，観察開始時年齢が40歳から79歳までの地域住民であり，地方自治体（大府市および東浦町）の協力を得て，住民台帳から年齢・性別に層化した無作為抽出を行っ

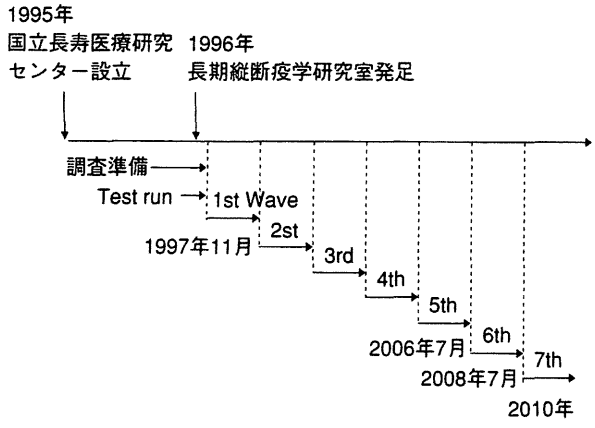

図1　国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）の経緯

NILS-LSAでは，地域在住の中高年者約2,400人の10年以上にわたるデータが蓄積されている．

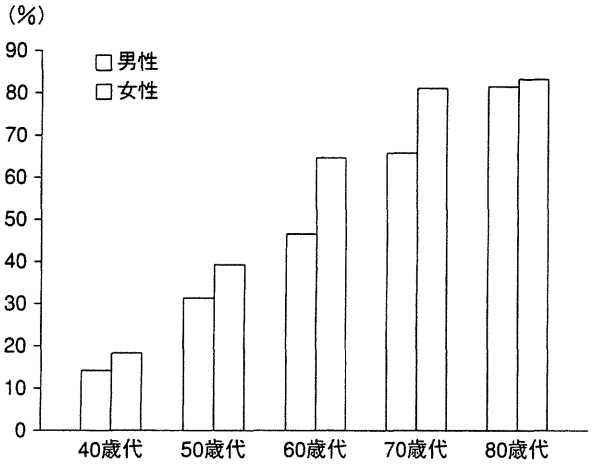

図3　年代別，性別の膝変形性関節症の有病率

Kellgren-Lawrence分類grade Ⅱ以上．

ている．抽出によって選定された者を説明会に招き，検査の目的や方法などを十分に説明し，インフォームドコンセントを得た上で検査を実施している．追跡中のドロップアウトは，同じ人数の新たな補充を行い，定常状態として約2,400人のダイナミックコホートを目指している．

検査は施設内に設けられた専用の検査センターで，朝9時から夕方4時までの間に分刻みでスケジュールを組んで行っている．調査項目は頭部MRIや超音波断層，骨密度測定，腹部CTなど最新の機器を利用した医学検査のみならず，詳細な生活調査，栄養調査，運動機能調査，心理検査など，広汎で精度の高い内容である（図2）．

運動器疾患に関連した検査としては，二重エネルギーX線吸収法（DXA）による全身骨，腰椎，左右大腿

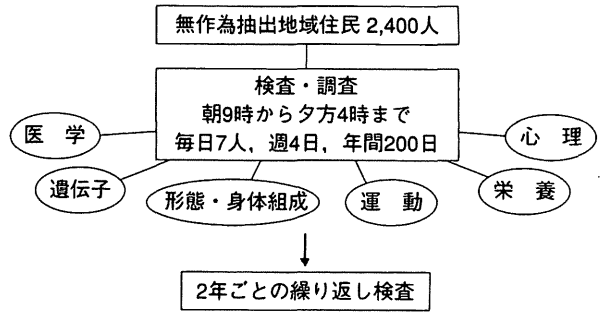

図2　国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）の概要

骨頸部の4スキャンでの骨密度測定，末梢骨定量CT検査法（pQCT）による橈骨16スキャン，左右膝X線撮影，胸椎腰椎X線撮影，膝関節機能検査，転倒調査，膝痛調査，腰痛調査，骨折調査，骨代謝マーカー検査などを実施している．また，調査開始当初より，調査参加者のほぼ全員からの血液サンプルを用いてDNAを蓄積している．これほど背景因子が詳細に検討されている一般住民のDNA試料の蓄積は，国内外でもほかにはほとんどないと思われる[7,8]．

加齢に伴う運動器疾患罹患の実態

加齢に伴う運動器疾患の実態を明らかにするためには，運動器疾患の有病率，日本人全体での推計患者数を求める必要がある．こうした推計は，無作為抽出された地域住民での調査によって実施されなければならない．老人会や運動教室などで募集したボラティアなどによる調査では，対象者の偏りが大きくなってしまい，正確な推計はできない．われわれは，地域住民から年齢・性別に層化した無作為抽出されたコホートであるNILS-LSAのデータを使って，運動器疾患の性別・年齢別の有病率を求めた．

変形性膝関節症について，NILS-LSAの第5次調査に参加した40歳から88歳の男性1,200人，女性1,219人の合計2,419人を対象として検討を行った．立位にて撮影された両膝のX線写真から，Kellgren-Lawrence分類（KL分類）[9]にて，膝関節の変形をgrade 0からgrade Ⅳまでに分類し，grade Ⅱ以上を変形性膝関節症と診断した．また，grade Ⅲ以上を膝関節高度変形として，10歳ごとの年齢別および性別に有病率を算定した．図3に示すように，変形性膝関節症は男性よりも女性に多く，年齢とともに有病率は上昇している．40歳以上の女性全体での有病率は52.3%，男性で43.5%であった．

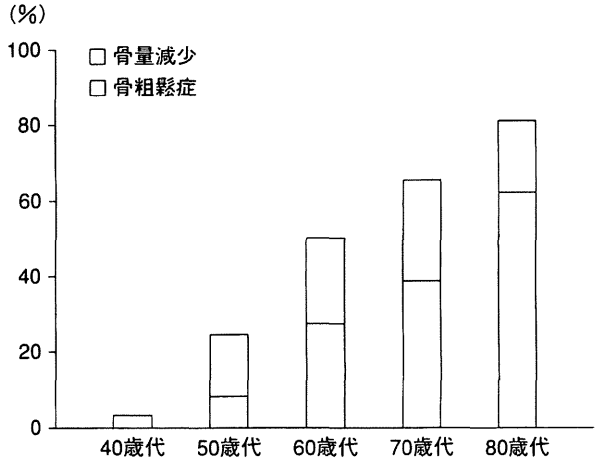

図4　女性の年代別の骨粗鬆症および骨量減少の有病率
日本骨代謝学会診断基準による腰椎骨密度からの判定.

また，KL分類grade Ⅲ以上の膝高度変形保有率は，女性の方が男性よりも2倍以上多く，また女性では年齢とともにその率は大きく上昇していた．上記の有病率を用いて日本人全体の人口構成から有病率を計算すると，男性1,278万人，女性1,950万人の合計3,228万人と推定された．

骨粗鬆症については日本骨代謝学会の診断基準[10]を用いて，DXAで計測した腰椎骨密度(第2，3，4腰椎の平均骨密度)，および右大腿骨頸部骨密度により診断し，性別，年齢別に骨粗鬆症の有病率を算定した(図4)．50歳以上の女性の有病率は，腰椎骨密度の判定の場合26.1%，大腿骨頸部骨密度による判定の場合には21.3%であった．骨粗鬆症/骨量減少の年代別有病率は，どちらの部位の判定でも，加齢とともに高くなり，特に60歳代で急激に高くなっていた．腰椎に比べ，大腿骨頸部判定の場合，50，60歳代での有病率は低かった．50歳以上の男性の骨粗鬆症有病率は，腰椎骨密度の判定の場合7.6%，大腿骨頸部骨密度判定の場合，10.3%であった．骨粗鬆症/骨量減少の年代別有病率は，大腿骨頸部の判定において，60歳代以降，男性でも加齢で高くなっていた．今回得られた骨粗鬆症有病率から，見積もられる骨粗鬆症患者数は，腰椎骨密度による有病率を用いると，50歳以上の女性で約811万人，50歳以上の男性で189万人と推計され，大腿骨頸部では，女性685万人，男性250万人であった．男女合計で骨粗鬆症患者数は900～1,000万人と推定された．

運動器疾患のリスク推定と予防

運動器疾患には生活習慣や疾病，遺伝などの様々な要因がある[11]．また，骨粗鬆症などでは，リスクを血液や尿のマーカー検査で予測しようとする試みがなされている．こうしたリスク要因やマーカーから，運動器疾患罹患のリスク推定ができる．

NILS-LSAでは頸部超音波断層検査を行い，動脈硬化の判定を頸動脈内膜中膜複合体厚(intima-media thickness：IMT)を用いて判定している．IMTを左右総頸動脈および左右頸動脈分岐部で計測し，その最肥厚部をIMTの代表値とした．IMTが1.1 mm以上を異常肥厚とし，動脈硬化ありと判定した．IMTによる動脈硬化の判定データと腰椎骨密度のデータを用いて，両疾病間の関連について解析を行った．その結果，女性で動脈硬化のある者は，ない者に比べて骨粗鬆症有病の割合が約2倍に高くなっていた[12]．

骨代謝マーカー測定によって，骨粗鬆症や骨量減少の予測ができるかをNILS-LSAのデータで検討した．骨代謝マーカーとしてオステオカルシン(OC)，骨型アルカリフォスファターゼ(BAP)，尿中Ⅰ型コラーゲン架橋N-ペプチド(NTx)，デオキシピリジノリン(DPD)を測定したところ，女性の腰椎でOC，BAP，NTxが，女性の大腿骨頸部でDPD，BAP，NTx，男性の大腿骨頸部でBAPが6年後の骨粗鬆症や骨量減少の発症に有意に関連しており，これらのマーカーから将来の骨粗鬆症や骨量減少の発症を予測できる可能性が示された[13]．

NILS-LSAでは，生活習慣や環境要因を考慮した骨粗鬆症の遺伝要因の検討も順次進めている．DXAによる骨粗鬆症診断結果と，握力，脚筋力など運動・体力に関する要因，カルシウム，ビタミンDなど栄養に関する要因，BMI，除脂肪体重など体格・体型に関する要因，その他，嗜好，閉経，骨代謝マーカーを含む血液尿検査結果などの項目の追跡による縦断的なデータについて，網羅的に検討を行うことで，それぞれ骨粗鬆症と関連の強い要因を探索する．さらに，抽出された要因と遺伝子多型との相互作用を網羅的に検討し，その結果から，最終的について骨粗鬆症と関連する生活習慣要因，遺伝子多型，生活習慣要因と遺伝子多型の交互作用を抽出し，骨粗鬆症の予測を行う総合的なシステムの構築を行っている．長期縦断研究により，こうしたシステムが完成すれば，骨粗鬆症の医療や予防の実用化へ一歩前進することができるものと期待される．

おわりに

日本人の平均寿命は年々延びて，高齢者の人口は増加を続けている．今後は，高齢者の健康を守ることがますます重要になってくる．高齢者の運動器疾患の予防と治療は，高齢者の健康長寿を考える場合には欠かすことができない．運動器疾患罹患のリスクを予想し，予防を行う方法を確立して行く必要がある．そのためのエビデンスを集積する研究として，疾患そのものだけでなく，遺伝子や栄養，運動までを含めた学際的な長期縦断疫学研究の進展が望まれる．

文献

1）Yoshimura N, Muraki S, Oka H, et al：Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women：the research on osteoarthritis/osteoporosis against disability study. J Bone Miner Metab 2009；**27**：620-628.
2）下方浩史：加齢研究の方法―横断的研究と縦断的研究．新老年学改訂第3版(大内尉義・秋山弘子編)，東京大学出版会，東京，2010；pp. 333-346.
3）Shimokata H, Ando F, Niino N：A new comprehensive study on aging-the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 2000；**10**：S1-S9.
4）下方浩史：長期縦断研究の目指すもの．Geriatric Medicine 1998；**36**：21-26.
5）下方浩史：老化および老年病の疫学的研究．Geriatric Medicine 2007；**45**：13-17.
6）下方浩史，安藤富士子：長期縦断疫学で分かったこと．日本老年医学会雑誌 2008；**45**：563-572.
7）下方浩史，藤澤道子，安藤富士子：老化・老年病の分子疫学．Molecular Medicine 2002；**39**：576-581.
8）下方浩史，安藤富士子：疾患ゲノム研究の現況：骨粗鬆症．Clinical Calcium 2008；**18**：155-161.
9）Kellgren JH, Lawrence JS：Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1956；**15**：1-11.
10）日本骨代謝学会骨粗鬆症診断基準検討委員会：原発性骨粗鬆症の診断基準(2000年度改訂版)．日本骨代謝学会雑誌 2001；**8**：76-82.
11）安藤富士子，下方浩史：臨床面接で把握する骨粗鬆症の危険因子：疫学研究の成果を生かして．Medicina 2008；**45**：430-433.
12）竹村真里枝，安藤富士子，下方浩史ほか：地域在住中高者年の骨代謝マーカーによる骨量減少/骨粗鬆症予測．Osteoporosis Japan 2007；**15**：28-32.
13）竹村真里枝，安藤富士子，下方浩史ほか：一般住民における動脈硬化と骨粗鬆症の関連．Osteoporosis Japan 2010；**18**：228-231.

Longitudinal Epidemiological Study on Locomotor Disease

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It is important to clarify the risk factors of locomotor disease for the investigation of locomotor disease prevention. By longitudinal studies in general population, it will be possible to elucidate the actual condition and prevalence of locomotor disease, and to figure out the association of nutrition, physical activity, coexisting illness, life-style including alcohol drinking and smoking, and genetic factors with development in age-related locomotor disease. A longitudinal study of aging at the National Center for Geriatrics and Gerontology (NILS-LSA) started in 1997 as a comprehensive epidemiological study in a randomly selected community-living population to collect basic data on aging and geriatric diseases. Using the NILS-LSA data, the number of patients with osteoporosis is estimated to be 10 million and the number of patients with knee osteoarthritis is estimated to be 30 million in the entire area of Japan. Now, we are advancing the research to clarify the risk factors of locomotor diseases and to develop preventive method in the elderly by longitudinal analysis on variables including gene, life-style, physical strength, and nutrition in the NILS-LSA.

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ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Spatiotemporal components of the 3-D gait analysis of community-dwelling middle-aged and elderly Japanese: Age- and sex-related differences

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Aim: To describe age- and sex-related differences in gait patterns of community-living men and women using 3-D gait analysis.

Methods: Subjects ($n = 2006$) aged 40–84 years participated in the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA). Spatiotemporal components, including velocity, step length, step frequency, and double support time during a gait cycle, were calculated from 3-D coordinates and vertical force data. Velocity, step length and step frequency were normalized by leg length and acceleration due to gravity, and double support time was normalized to gait cycle duration.

Results: Spatiotemporal walking variables of brisk velocity and step length were significantly greater in men than in women, while comfortable velocity and comfortable and brisk step frequencies and double support times were greater in women than in men. Age-related changes were marked at 70–84 years in most spatiotemporal variables in both sexes during comfortable walking. During brisk walking, age-related changes were observed from a younger age than during comfortable walking, and there were sex-related differences.

Conclusion: The age-related gait alteration was obvious among those aged 70 years and older, and it accelerated markedly in women's brisk walking intensity. **Geriatr Gerontol Int 2011; 11: 39–49.**

Keywords: aging, gait, sex, velocity, walking.

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Author contributions: W. D. designed the study, obtained the funding, analyzed data and drafted the original article; R. K. interpreted data and advised on revising the article; K. H. Y. supervised data processing and prepared the article; and F. A. and H. S. originated the study, created the gait analysis program, supervised all aspects of its implementations, and contributed to obtaining the funding and revising the article. All authors conducted epidemiological studies on geriatric disease and human aging in Obu, Aichi, Japan, and read and approved the manuscript.

Introduction

Age-related impairment of ambulatory ability is a critical component for inhibiting activities of daily living (ADL). For instance, decreased gait velocity observed in elderly is an indicator of common distinct diseases[1,2] and falls,[3–6] which lead to functional dependence[7–11] or death.[12] The prevalence and incidence of gait disorders increase with age in elderly persons.[13,14] The early presence of dynamic postural stability may provide more essential information for preserving adequate mobility, delaying the onset of functional decline and encouraging early appropriate lifestyle changes to promote active healthy aging.[6,8,10,11,15]

Previous studies examined age-related changes in spatiotemporal gait parameters including velocity, step length, step frequency (cadence) and selected stride time variables (single and double support time and swing time).[7,8,10,16–21] These performance-based gait variables were often measured by a 3-D gait system that computes the motions of the body center of mass (COM) and each segment, which can accurately evaluate the control of dynamic balance during walking.[22,23] The COM velocity on the 3-D gait system identified the effect of age on older gait in limited comparison between young and older groups.[24–26] It showed that the 3-D analyses conducted have not determined from which age group the accelerated decline of gait started. The collection of data using a large sample size with a broad age range could resolve the issue.

Age-related gait studies have recruited either men or women, or both sexes have been analyzed together: a few studies previously focused on sex-related changes on gait pattern with advancing age. Callisaya *et al.*[8] revealed the effects of sex and age on gait velocity in elderly men and women aged 60–86 years. The results of other studies of various age ranges and groups[17,19,27] to determine which sex shows an earlier age of accelerated gait velocity decrease have differed. The conflicts may partly depend on the sampling and subject characteristics.

Therefore, to understand the aging process in gait measures across the adult lifespan, a large sample size ranging from young or middle-aged to elderly men and women should be warranted. We decided to reinvestigate the previous findings. In the present study, the gait of elderly subjects was investigated based on comfortable and brisk spatiotemporal gait parameters with a 3-D gait analysis system; a large number of subjects were recruited. We found the age-related changes in gait by sex among middle-aged and elderly men and women in Japan. This may contribute to a beneficial effect on assessing gait in elderly people and making an adequate walking exercise program suitable for targeted age groups.

Methods

Study sampling

The present gait analysis is part of the third phase of the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA); this study includes medical, physiological, nutritional and psychological examinations. The study began in November 1997 (the first phase), and the third phase lasted from May 2002 to May 2004. The subjects were age- and sex-stratified random samples of the population, aged 40–84 years, who lived in Obu-shi and Higashiura-cho, Aichi, Japan. These participants were chosen from the residents registered with local governments. All subjects lived or had lived at their home in the community and had Japanese nationality.[28] The NILS-LSA was approved by the Ethics Committee of the National Center for Geriatrics and Gerontology. Details of the NILS-LSA have been previously published.[28,29]

Of 2378 men and women aged 40–84 years in the third phase examination, 1017 men and 989 women (84.2% of all participants, Table 1) completed the walking tests and were included in the present analysis. The participants also completed a structured questionnaire dealing with their socioeconomic characteristics, cardiovascular risk factors and medical history.[28,29] Exclusion criteria included a current medical history of arthritis[6,8] and fractures (musculoskeletal disorders),[30] stroke[1] and Parkinson's disease (neurological disorders),[8,31] and ischemic heart disease and chronic bronchitis (Table 1).[32,33] These diseases were checked and excluded as the possible cause of gait disorders or spatiotemporal gait parameter changes by a physician before the walking tests. One participant who was diagnosed with dementia was excluded because she had a limited ability to comprehend or execute the test, which was judged by a physician. The existence of walking difficulty in activities of daily living (ADL)[11,15] was also excluded (Table 1). The participants who met the above-mentioned requirements and could walk 10 m independently without a walking aid were included in the current gait analysis and therefore 372 participants of the third phase examination were totally excluded.

Protocol

All participants wore short-sleeved T-shirts and shorts for testing. Shoes were made from the same material that had a vinylon/polyester and cotton blended upper part and a urethane foam outsole (Moonstar, Fukuoka, Japan), and were selected to exactly fit each participant's feet. Ten 2.5-cm diameter optical markers were placed on the participants' left and right sides on the fifth metatarsal heads, the lateral malleoli, the lateral epicondyles, and one-third of the way along the straight lines from the greater trochanters to the anterior

Table 1 Inclusion/exclusion characteristics of 2378 participants in the third wave examination of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), 2002–2004

Characteristics	Men	Women
Inclusion (n = 2006)		
Total (n (%))	1017 (50.7)	989 (49.3)
Age group (n (%))†		
40s	250 (12.5)	279 (13.9)
50s	302 (15.1)	265 (13.2)
60s	250 (12.5)	242 (12.1)
≥70	215 (10.7)	203 (10.1)
Exclusion (n = 372)		
Total (n (%))	187 (50.3)	185 (49.7)
Prevalence of disease (n (%))		
Stroke	42 (22.5)	23 (12.4)
Ischemic heart disease	41 (21.9)	41 (22.2)
Chronic bronchitis	7 (3.7)	3 (1.6)
Arthritis	26 (13.9)	56 (30.3)
Fracture	5 (2.7)	6 (3.2)
Dementia	–	1 (0.5)
Parkinson's disease	3 (1.6)	–
Walking difficulties in ADL (n (%))	50 (26.7)	54 (29.2)
Not completed walking test (n (%))	55 (29.4)	53 (28.6)

†χ^2-Test test examines significance among each age group and sex. Values are numbers (% of total at each inclusion/exclusion category) of samples. ADL, activities of daily living.

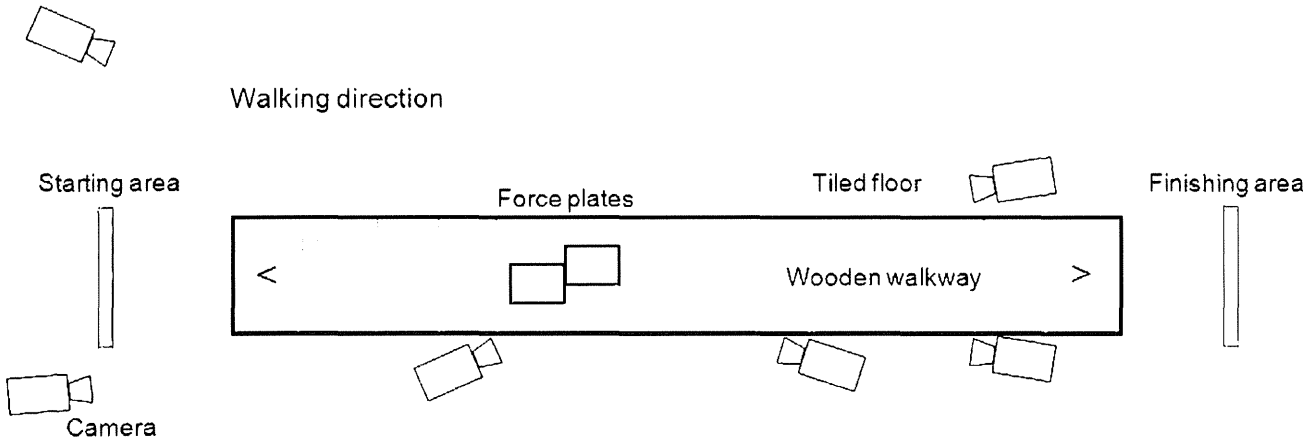

Figure 1 Setup of 3-D gait system: the 10-m walkway consisted of a wooden walkway. Six cameras were placed at various positions and two force platforms were embedded in the center of the walkway. Double support time in pre-swing phase of right foot was measured in this setting.

superior iliac spines and the acromions.[34] The subjects walked on a 10-m walkway at two speeds: (i) at a self-selected pace (comfortable walking); and (ii) as fast as possible without running (brisk walking). Each pace was repeated approximately twice on average. The walkway consisted of a tiled floor and a wooden walkway along the corridor (Fig. 1). The surface of the wooden walkway was covered with gray-colored, thin, stiff rubber, which measured 0.036 m in height from the tile floor surface of the corridor. Force platforms (0.6 m × 0.4 m) (9286; Kistler Instrumente AG, Winterthur, Switzerland), with surface colors similar to those of the walkways, were embedded in the center of the wooden walkway. The starting point for each trial was

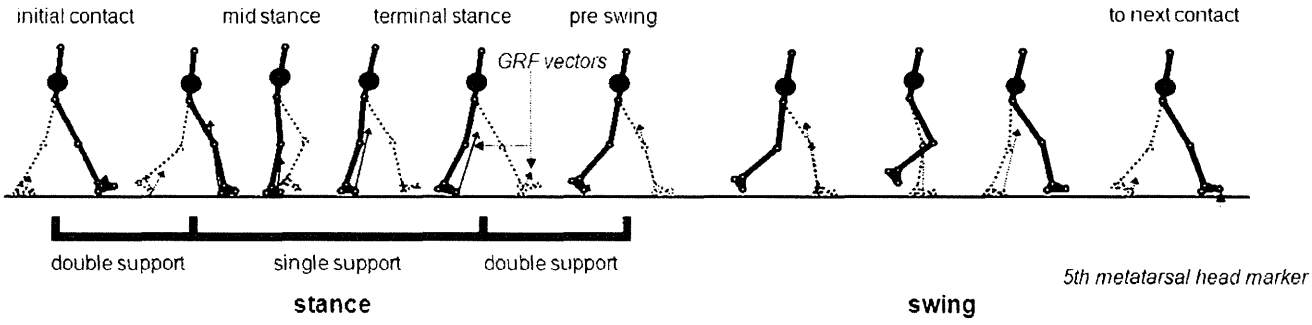

Figure 2 Definition of gait cycle using ground reaction force (GRF) and the fifth metatarsal head marker.

selected in relation to the foot contacts on the force platforms. The distance from each starting and departure point to the force platforms was approximately 3.5–4.5 m. One trial each of comfortable and brisk walking was used in the data analysis. The trials used were those that lacked the least data.

The Vicon 370 system (Oxford Metrics Ltd, Oxford, UK), which consisted of six cameras, was used to obtain the 3-D coordinates of the trunk, thighs, shins and feet. The calibration residual at each camera was set below 1.0 mm. The data were processed using a custom routine that was programmed by the Clinical Gait Analysis Forum of Japan.[34] The raw coordinate data at 60 Hz were digitally filtered with a fourth-order, zero-lag, Butterworth filter[22] with a cut-off at 5 Hz, and the raw ground reaction force data at 1200 Hz were digitally filtered with a cut-off at 10 Hz. The force data were interpolated to correspond with the coordinate data to synchronize the datasets. Smoothed coordinates of the lower extremities were used to construct a rigid link-segment model.[22] Segment masses and inertial properties were determined using previously reports[35] and the participants' mass and height, which were used for calculating COM.

Gait cycle and walking variable calculation

SAS ver. 9.1.3.[36] was used to automatically identify gait event times and each phase of the gait cycle based on kinematic and kinetic gait data. The divisions of the gait cycle are shown in Figure 2.[30] The gait event times for initial contacts and toe off were determined using vertical force data and the vertical motion of the optical marker on the fifth metatarsal head. The period from the first right initial contact to ipsilateral second initial contact was one gait cycle.[30]

Both the right and left leg motions were captured, and primarily the right stride was analyzed. Left leg motion was used for calculating the step length and double support times. The mean COM velocities, step lengths, step frequencies and double support times during a gait cycle were also automatically computed by SAS. The double support time was defined as the duration of time during which each foot was on the ground in the pre-swing phase. The mean COM velocity, step length, and step frequency were normalized as proposed by Hof[37] as follows:

Normalized COM velocity, $\hat{v} = \frac{v}{\sqrt{gl_0}}$,

Normalized step length, $\hat{l} = \frac{l}{l_0}$,

Normalized step frequency, $\hat{f} = \frac{f}{\sqrt{g/l_0}}$,

where v is actual mean COM velocity, l_0 is the leg length of each subject, l is the actual step length, f is the actual step frequency and g is the acceleration due to gravity (9.81 m/s^2). Leg length was measured from the ground to the greater trochanter during quiet standing. Patients with arthritis and fracture were excluded (Table 1), and no case of limited knee extension was observed in the present study. The double support time was also normalized by each subject's cycle duration, from right initial contact to next right initial contact (over one gait cycle).

For the calculation of walking variables, technical difficulties sometimes caused missing data due to the effect of occlusion while capturing motion. Thus, for example, the mean COM velocity over the gait cycle was calculated using data from 1716 men and women (85.5% of the total sample) during comfortable walking and using data from 1614 men and women during brisk walking (80.4%). To demonstrate the lack (or presence) of bias with respect to velocity data loss, the Student's t-test was used to compare the velocity between the group with all available data and that with data available only in the velocity category. The results showed that the velocities were not significantly different between the two groups, and this was confirmed for all walking variables.

Statistical analyses

All analyses were performed using SAS ver. 9.1.3. Sex differences were examined using the Student's t-test. For analysis of age differences, participants were divided

into eight groups based on sex and age (40–49, 50–59, 60–69 and 70–84 years for each sex). Trends in differences across all age groups in the walking variables were tested using the General Linear Model (GLM), and differences by age group were tested using the Tukey–Kramer method for each sex. $P < 0.05$ was considered statistically significant.

Results

The proportion of the sample drawn from each age group and each sex group was the same (χ^2-test, $P > 0.05$). The mean ± standard deviation age was 58.1 ± 11.4 years in men and 58.7 ± 11.4 years in women, which was not significant ($P > 0.05$).

The results of the GLM and Tukey–Kramer tests revealed age-related changes in each age and sex group. Descriptive statistics for all values are shown in Tables 2 and 3 and Figure 3. Mean COM velocities during comfortable and brisk walking significantly decreased with age in both sexes ($P < 0.001$). Age-related changes in the comfortable COM velocity were marked in the 70–84-year group compared with other age groups. Similar changes were found in the brisk COM velocity. The step lengths and frequencies followed these COM velocity patterns in both sexes during both comfortable and brisk walking.

These age-related changes occurred earlier in the middle-aged group. Earlier patterns involving brisk gait parameters were more apparent in women: for example, the brisk COM velocity decreased at 60–69 years in men and at 50–59 years in women, then the decrease accelerated at 70–84 years (Tables 2,3, Fig. 3). The step length and frequency followed these COM velocity patterns. The double support time during pre-swing was significantly increased with age only at the women's comfortable walking pace; it was significantly longer in the 70–84-year group compared to other age groups (Table 3, Fig. 3). The men's double support times showed no significant age-related differences among age groups (P for trend >0.05, Fig. 3).

Descriptive statistics and the results of sex differences for gait parameters are depicted in Table 4. The results of mean COM velocity differed according to walking pace: the comfortable COM velocity was significantly faster in women than in men ($P < 0.001$), and the brisk COM velocity was significantly faster in men than in women. Step length pattern was similar to COM velocity pattern: the brisk step length was longer in men than in women ($P < 0.001$), but the comfortable step length was not significantly different. On the other hand, women had a higher step frequency during both walking paces ($P < 0.001$). The results of the pre-swing double support time were equal to the step frequency.

Discussion

Mobility is essential for independence in the elderly. A better understanding of age-related changes in gait provides useful information for appropriate intervention programs targeting specific age groups.[8] The present cross-sectional, descriptive study showed spatiotemporal components of gait over one gait cycle among community-living middle-aged and elderly Japanese subjects. The sample of 1017 men and 989 women was large enough to allow analysis by age group,[17] and, to the best of our knowledge, the sample size is the largest to be published in which gait characteristics have been analyzed using a 3-D gait system. There was no disproportionate lack of gait data caused by difficulties in capturing the 3-D coordinates.

Mean COM velocities decreased with age, which is in almost complete agreement with previous results, despite the use of different measurement equipment and instrumentation.[16–21,25,29] The age-related decreases in the normalized COM velocities accelerated at 70 years and over were noted at a relatively later age compared with the previous reports: they showed the accelerated decline occurred in 50–59- and 60–69-year age groups,[17] at 62 years,[19] between 60- and 70-year age groups,[20] and at 65 years and in the 67–73-year age group.[18] The differences in age of accelerated decline among the previous and the present findings were likely due to the differences in method and data characteristics.

The brisk COM velocity decreases advancing with age were earlier compared with the comfortable walking. Some previous studies showed the age-related decrease was independent of walking pace,[18–20] while another reported that the decrease depended on the pace.[7] In a report by Bohannon on the comfortable and maximum walking speeds of adults aged 20–79 years,[7] walking speed was found to be influenced by the interaction of pace and age. This result matched our present findings that the age-related decrease was clearer during brisk walking than during comfortable walking. Moreover, these earlier age-related declines in the brisk COM velocities were apparent in women. Some studies reported that the critical age for marked velocity decrease did not differ by sex,[16,19] while another found the critical age to be earlier in men.[17] However, Callisaya *et al.*[8] showed women's walking velocity to be an earlier age-related change compared to men's parameters during the preferred speed of walking among the subjects aged 60 years and older. These results are in agreement with our own, though our data was particularly strong in the brisk parameters across middle-aged and elderly persons. The brisk walking task required greater forward momentum and increased demands in muscle activity[24,38–40] and aerobic capacity[33,41] might alter the spatiotemporal gait parameters accompanying aging.

Table 2 Men's normalized mean COM velocities, step lengths and frequencies and double support times during comfortable and brisk walking in each age group

Men: walking parameters by age group	Mean COM velocity				Step length				Step frequency				Double support times (pre-swing)			
	N	Mean	SD	95% CI	N	Mean	SD	95% CI	N	Mean	SD	95% CI	N	Mean	SD	95% CI
Comfortable walking																
40s	211	0.524	0.053	0.517–0.531	240	0.892	0.065	0.884–0.900	207	0.587	0.043	0.582–0.593	208	14.8	1.5	14.6–15.0
50s	266	0.527	0.059	0.520–0.534	289	0.897	0.076	0.888–0.906	259	0.590	0.042	0.585–0.595	249	14.8	1.5	14.6–14.9
60s	218	0.523	0.067	0.514–0.532	240	0.901	0.089	0.890–0.913	215	0.583	0.046	0.577–0.589	205	14.5	1.6	14.3–14.7
70–	186	0.485	0.070	0.475–0.495	213	0.859	0.096	0.846–0.872	185	0.569	0.047	0.562–0.576	177	15.2	2.0	14.9–15.5
P for trend†	<0.001				<0.001				<0.001				NS			
(Tukey–Kramer test)‡	40s, 50s, 60s >70–				40s, 50s, 60s >70–				40s, 50s, 60s >70–				NA			
Brisk walking																
40s	190	0.705	0.078	0.694–0.716	229	0.998	0.074	0.989–1.008	180	0.707	0.070	0.696–0.717	173	13.3	6.0	12.4–14.2
50s	235	0.699	0.082	0.688–0.709	272	0.998	0.088	0.987–1.008	214	0.697	0.064	0.688–0.705	209	13.3	5.6	12.6–14.1
60s	191	0.678	0.079	0.667–0.690	237	1.000	0.094	0.988–1.012	185	0.685	0.066	0.676–0.695	180	13.4	5.0	12.6–14.1
70–	182	0.618	0.092	0.605–0.631	203	0.946	0.100	0.932–0.960	177	0.657	0.066	0.647–0.667	169	14.1	2.1	13.8–14.4
P for trend†	<0.001				<0.001				<0.001				NS			
(Tukey–Kramer test)‡	40s > 60s > 70–., 50s > 70–				40s, 50s, 60s >70–				40s > 60s > 70–., 50s > 70–				NA			

†Trend tests examine main effects of age in each gait parameter. ‡Tukey–Kramer tests examine the significant difference among each age group. '>' indicates the significant difference between the age groups, with *P*-value is less than 0.5. Values are numbers of samples (N), means (Mean), standard deviations (SD) and 95% confidence intervals (95% CI) at each variable. Age group: 40s, 40–49 years age group; 50s, 50–59 years age group; 60s, 60–69 years age group; 70–, 70–84 years age group. COM, center of mass; NS, not significant; NA, not applicable.

Table 3 Women's normalized mean COM velocities, step lengths and frequencies and double support times during comfortable and brisk walking in each age group

Women: walking parameters by age group	Mean COM velocity				Step length				Step frequency				Double support times (pre-swing)			
	N	Mean	SD	95% CI	N	Mean	SD	95% CI	N	Mean	SD	95% CI	N	Mean	SD	95% CI
Comfortable walking																
40s	228	0.542	0.060	0.535–0.550	267	0.905	0.072	0.896–0.913	223	0.602	0.044	0.596–0.608	212	14.9	1.7	14.7–15.2
50s	224	0.547	0.066	0.538–0.556	252	0.902	0.082	0.891–0.912	219	0.607	0.051	0.600–0.614	214	14.9	1.7	14.7–15.1
60s	210	0.536	0.064	0.527–0.544	236	0.890	0.079	0.880–0.900	207	0.602	0.045	0.596–0.608	189	15.0	1.9	14.8–15.3
70–	173	0.472	0.071	0.461–0.483	189	0.833	0.093	0.820–0.847	169	0.570	0.051	0.562–0.578	148	15.8	1.9	15.5–16.1
P for trend†	<0.001				<0.001				<0.001				<0.001			
(Tukey–Kramer test)‡	40s, 50s, 60s >70–				40s, 50s, 60s >70–				40s, 50s, 60s >70–				70– > 60s, 50s, 40s			
Brisk walking																
40s	216	0.702	0.072	0.692–0.711	269	0.972	0.070	0.963–0.980	210	0.728	0.071	0.719–0.738	201	13.9	1.6	13.7–14.2
50s	215	0.675	0.080	0.665–0.686	252	0.960	0.087	0.950–0.971	212	0.706	0.073	0.696–0.715	209	14.2	1.7	13.9–14.4
60s	212	0.653	0.072	0.643–0.662	230	0.941	0.085	0.929–0.952	209	0.696	0.072	0.687–0.706	199	14.2	1.8	14.0–14.5
70–	173	0.577	0.084	0.565–0.590	187	0.890	0.109	0.875–0.906	163	0.651	0.064	0.562–0.578	157	14.3	8.8	12.9–15.7
P for trend†	<0.001				<0.001				<0.001				NS			
(Tukey–Kramer test)‡	40s > 50s > 60s > 70–				40s > 60s > 70–., 50s > 70–				40s > 50s, 60s > 70–				NA			

†Trend tests examine main effects of age in each gait parameter. ‡Tukey–Kramer tests examine the significant difference among each age group. '>' indicates the significant difference between the age groups, with $P < 0.05$. Values are numbers of samples (N), means, standard deviations (SD) and 95% confidence intervals (95% CI) at each variable. Age group: 40s, 40–49 years age group; 50s, 50–59 years age group; 60s, 60–69 years age group; 70–, 70–84 years age group. COM, center of mass; NS, not significant; NA, not applicable.

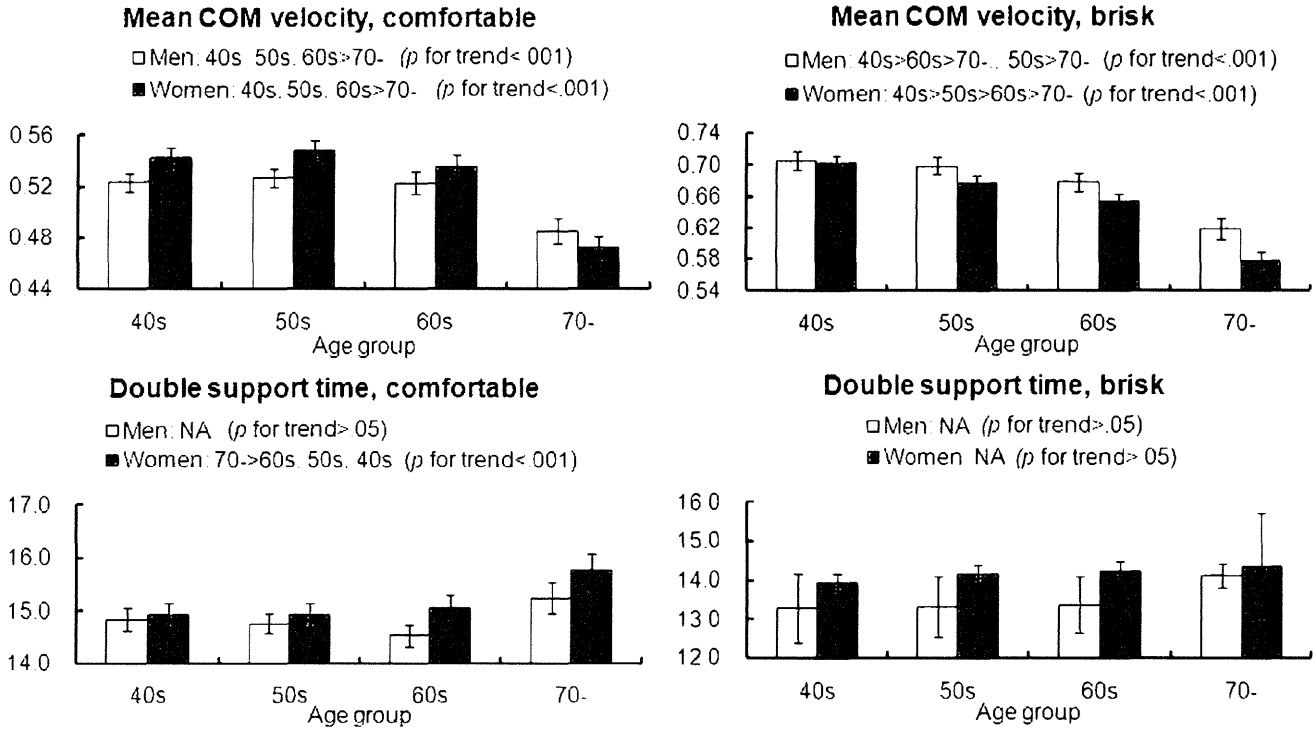

Figure 3 Age-related differences (trend tests and Tukey–Kramer tests); means and 95% confidence intervals of normalized mean center of mass (COM) velocities ($(m/sec)/\sqrt{((m/sec^2)\times m)}$) and double support times (s/s) during comfortable and brisk walking in men and women. Significant differences by age group in men and women are noted on the upper side of each figure. '>' indicates the significant difference between the age groups, with *P*-values of ≤0.05.

Table 4 Normalized mean COM velocities, step lengths and frequencies and double support times during comfortable and brisk walking among men and women

Walking parameters	Men				Women				*P*-value†
	N	Mean	SD	95% CI	N	Mean	SD	95% CI	
Comfortable walking									
Mean COM velocity	881	0.516	0.064	0.512–0.521	835	0.527	0.071	0.523–0.532	<0.001
Step length	982	0.889	0.083	0.883–0.894	944	0.886	0.085	0.881–0.891	NS
Step frequency	866	0.583	0.069	0.580–0.586	818	0.597	0.045	0.593–0.600	<0.001
Double support time (pre-swing)	839	14.8	1.7	14.7–14.9	763	15.1	1.8	15.0–15.2	<0.001
Brisk walking									
Mean COM velocity	798	0.677	0.089	0.671–0.683	816	0.656	0.089	0.650–0.662	<0.001
Step length	941	0.987	0.092	0.981–0.993	938	0.945	0.092	0.939–0.951	<0.001
Step frequency	756	0.687	0.075	0.682–0.692	794	0.698	0.049	0.693–0.703	<0.001
Double support time (pre-swing)	731	13.5	5.0	13.2–13.9	766	14.2	4.3	13.9–14.5	<0.01

†Student *t*-tests examine the sex differences. Values are numbers of samples (N), means, standard deviations (SD) and 95% confidence intervals (95% CI) at each variable. COM, center of mass; NS, not significant.

Further investigation should have discussed the difference between comfortable and brisk walking parameters.[38,42,43]

Age-related step length decreases during comfortable and brisk walking were almost concomitant with the COM velocity decreases, which was similar to the previous findings.[16,20] In brisk walking, however, age-related reduction in the step length seemed to be smaller than that in the step frequency compared with comfortable walking. For example, women's brisk step length decrease was 8.4% across middle-aged and elderly groups compared with their step frequency decrease of 10.7% (Table 3). This was observed also in men's. This may suggest that ambulatory ability observed in the COM velocity may be caused more by the step length during comfortable walking and the step frequency

during brisk walking in the elderly. This was also apparent in middle-aged women. The interpretation was limited qualitatively and should be further explored.

Step frequencies also decreased with age and this decrease was found even in middle-aged women during brisk walking. Previous studies in step frequency reported no age-related changes,[16,17,21] age-related decrease[8,18–20,25] and age-related increase.[26] Moreover, the current age- and sex-related decrease depending on required walking pace was not previously reported.[16,17] One explanation of these conflicts was that degree of the age-related reduction in step frequency was relatively less than that in other gait parameters such as velocity or step length.[8,17,19,20] Therefore, sample size, subject characteristics and measuring instruments may affect the age-related decrease in the step frequency.[16,25] Double support times in the present study did not increase with age, with the exception of women's comfortable data. On the other hand, exploratory analyses of actual values of double support times showed age-related increases in both sexes during both walking paces (data not shown, P for trend <0.001, <0.022). This shows that the double support as a percentage of one gait cycle remained almost constant in middle-aged and elderly subjects. Ferrandez *et al.*[32] found that double support time increased as velocity decreased, and that prolonged double support time was affected more by walking velocity than age.

The present study found brisk COM velocity and step length to be greater in men than in women. By contrast, step frequencies and double support times were greater in women than in men. This is characteristic of sex differences and is supported by previous findings.[8,17,21] Although the comfortable COM velocity was faster in women than in men, this is believed to be a result of the difference in body size as the actual comfortable COM velocity was significantly faster in men than in women (men, 1.46 ± 0.18 m/s; women, 1.43 ± 0.20 m/s; $P < 0.001$). The comfortable step length did not differ significantly between either sex group, perhaps because of the slower men's COM velocity.

The present gait data may give some insight into gait assessment and preventive walking exercise programs for older persons as previously reported.[42,44,45] The values for the gait parameters during one gait cycle may be useful to clinicians judging the ambulatory ability of patients from a short indoor walk.[7,42] Patients whose gait parameters are lower than that of their appropriate age group are at increased risk of ADL difficulties.[8,11] Comfortable and brisk walking velocities are predictive of adverse outcomes such as loss of physical function, requirement of caregivers, hospitalization and increased mortality in elderly persons.[8,10–12] Decreased step length and prolonged double support time are correlated with fear of falling and/or future fall risk.[4,5,9] Also, the other gait parameters such as gait velocity,[9,11] stride-to-stride variability[4] and lateral sway[3,5,6,46] are associated with the falling events. We did not directly ascertain whether the participants had a history of falls and/or a fear of falling in our gait parameters. Further work should confirm which gait measure is the best independent predictor for future fall risk in a large sample.

A moderate workload prescription in walking exercise programs should be given by controlling both step length and step frequency during comfortable walking in the elderly. Brisk walking, which is recommended for moderately vigorous endurance training and has a high impact compared to comfortable pace walking, might be considered for middle-aged women and the elderly to improve physical functions such as muscle strength[7,40,43] and/or cardiovascular fitness.[33,41]

This study has some limitations. Some previous gait investigations used the results of several trials or mean values of gait, while we used gait data from one trial of each participant. This was done because of technical difficulties in the automatically computed 3-D gait parameters. Next, the conjunction of our excluding criteria with the potential diseases might overestimate gait disorders: the elderly subjects were more likely to be healthy and physically fit. Moreover, patients with dementia were considered to be less in the present sample. The general comparability of the present gait variables with previously reported data is limited because of the lack of data for young adults in their 20s and 30s. Furthermore, our cross-sectional analysis approach could not demonstrate a cause-and-effect relationship from aging. We are planning longitudinal studies to further determine the effects of aging on gait. The present study included regional limitations such as race, culture, lifestyle, genetics and socioeconomic status which also may be important. However, the findings did permit age- and sex-related differences in gait to be clarified in the elderly.

In conclusion, age- and sex-related gait alterations were apparent in one gait cycle of walking in a large sample of community-dwelling, middle-aged and elderly Japanese men and women, when analyzed by a 3-D gait system. There were marked age-related gait differences in subjects aged 70 years and over compared to subjects aged 40–69 years during comfortable walking, and subtle differences were also observed in subjects aged 40–69 years during brisk walking. The earlier age-related changes were clearer in women than in men. These results may guide the assessment of gait patterns attributed to usual aging and to develop moderate exercise programs for the elderly.

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References

1 Bloem BR, Haan J, Lagaay AM, van Beek W, Wintzen AR, Roos RA. Investigation of gait in elderly subjects over 88 years of age. *J Geriatr Psychiatry Neurol* 1992; **5**: 78–84.
2 Marchetti GF, Whitney SL, Blatt PJ, Morris LO, Vance JM. Temporal and spatial characteristics of gait during performance of the Dynamic Gait Index in people with and people without balance or vestibular disorders. *Phys Ther* 2008; **88**: 640–651.
3 Tinetti ME, Speechley M, Ginter SF. Risk factors for falls among elderly persons living in the community. *N Engl J Med* 1988; **319**: 1701–1707.
4 Maki BE. Gait changes in older adults: predictors of falls or indicators of fear. *J Am Geriatr Soc* 1997; **45**: 313–320.
5 Barak Y, Wagenaar RC, Holt KG. Gait characteristics of elderly people with a history of falls: a dynamic approach. *Phys Ther* 2006; **86**: 1501–1510.
6 Chiba H, Ebihara S, Tomita N, Sasaki H, Butler JP. Differential gait kinematics between fallers and non-fallers in community-dwelling elderly people. *Geriatr Gerontol Int* 2005; **5**: 127–134.
7 Bohannon RW. Comfortable and maximum walking speed of adults aged 20–79 years: reference values and determinants. *Age Ageing* 1997; **26**: 15–19.
8 Callisaya ML, Blizzard L, Schmidt MD, McGinley JL, Srikanth VK. Sex modifies the relationship between age and gait: a population-based study of older adults. *J Gerontol A Biol Sci Med Sci* 2008; **63**: 165–170.
9 Verghese J, Holtzer R, Lipton RB, Wang C. Quantitative gait markers and incident fall risk in older adults. *J Gerontol A Biol Sci Med Sci* 2009; **64A**: 896–901.
10 Shinkai S, Watanabe S, Kumagai S *et al.* Walking speed as a good predictor for the onset of functional dependence in a Japanese rural community population. *Age Ageing* 2000; **29**: 441–446.
11 Montero-Odasso M, Schapira M, Soriano ER *et al.* Gait velocity as a single predictor of adverse events in healthy seniors aged 75 years and older. *J Gerontol A Biol Sci Med Sci* 2005; **60A**: 1304–1309.
12 Krishnamurthy M, Verghese J. Gait characteristics in nondisabled community-residing nonagenarians. *Arch Phys Med Rehabil* 2006; **87**: 541–545.
13 Verghese J, LeValley A, Hall CB, Katz MJ, Ambrose AF, Lipton RB. Epidemiology of gait disorders in community-residing older adults. *J Am Geriatr Soc* 2006; **54**: 255–261.
14 Berlau DJ, Corrada MM, Kawas C. The prevalence of disability in the oldest-old is high and continues to increase with age: findings from The 90+ Study. *Int J Geriatr Psychiatry* 2009; **24**: 1217–1225.
15 Demura S, Yamada T, Shin S. Age and sex differences in various stepping movements of the elderly. *Geriatr Gerontol Int* 2008; **8**: 180–187.
16 Oberg T, Karsznia A, Oberg K. Basic gait parameters: reference data for normal subjects, 10–79 years of age. *J Rehabil Res Dev* 1993; **30**: 210–223.
17 Auvinet B, Berrut G, Touzard C *et al.* Reference data for normal subjects obtained with an accelerometric device. *Gait Posture* 2002; **16**: 124–134.
18 Murray MP, Kory RC, Clarkson BH. Walking patterns in healthy old men. *J Gerontol* 1969; **24**: 169–178.
19 Himann JE, Cunningham DA, Rechnitzer PA, Peterson DB. Age-related changes in speed of walking. *Med Sci Sports Exerc* 1988; **20**: 161–166.
20 Kaneko M, Morimoto Y, Kimura M, Fuchimoto K, Fuchimoto T. A kinematic analysis of walking and physical fitness testing in elderly women. *Can J Sports Sci* 1991; **16**: 223–228.
21 Blanc Y, Balmer C, Landis T, Vingerhoets F. Temporal parameters and patterns of the foot roll over during walking: normative data for healthy adults. *Gait Posture* 1999; **10**: 97–108.
22 Winter DA. *Biomechanics and Motor Control of Human Movement*, 2nd edn. New York: John Wiley and Sons, 1991.
23 Pai Y-C, Patton J. Center of mass velocity-position predictions for balance control. *J Biomech* 1997; **30**: 347–354.
24 Riley PO, Della Croce U, Kerrigan DC. Effect of age on lower extremity joint moment contributions to gait speed. *Gait Posture* 2001; **14**: 264–270.
25 Prince F, Corriveau H, Hebert R, Winter DA. Gait in the elderly. *Gait Posture* 1997; **5**: 128–135.
26 Judge JO, Ounpuu S, Davis RB. Effects of age on the biomechanics and physiology of gait. *Clin Geriatr Med* 1996; **12**: 659–678.
27 Jansen EC, Vittas D, Helberg S, Hansen J. Normal gait of young and old men and women Ground reaction force measurement on a treadmill. *Acta Orthop Scand* 1982; **53**: 193–196.
28 Shimokata H, Ando F, Niino N. A new comprehensive study on aging – National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). *J Epidemiol* 2000; **10** (1 Suppl): S1–S9.
29 Kozakai R, Tsuzuku S, Yabe K, Ando F, Niino N, Shimokata H. Age-related changes in gait velocity and leg extension power in middle-aged and elderly people. *J Epidemiol* 2000; **10** (1 Suppl): S77–S81.
30 Perry J. *Gait Analysis: Normal and Pathological Function.* Thorofare, NJ: Slack, Inc., 1992.
31 Dobbs RJ, Charlett A, Bowes SG *et al.* Is this walk normal? *Age Ageing* 1993; **22**: 27–30.
32 Ferrandez A-M, Pailhouse J, Durup M. Slowness in elderly gait. *Exp Ageing Res* 1990; **16**: 79–89.
33 Tully MA, Cupples ME, Chan WS, McGlade K, Young IS. Brisk walking, fitness, and cardiovascular risk: a randomized controlled trial in primary care. *Prev Med* 2005; **41**: 622–628.
34 Clinical Gait Analysis Forum of Japan. *DIFF Manual*, ver. 1992.06, 1999.
35 Chandler RF, Clauser CE, McConville JT, Reynolds HM, Young JW. *Investigation of Inertial Properties of the Human Body.* Technical Report AMRL-TR-74-137. Dayton, OH: Wright-Patterson Air Force Base, 1975.
36 SAS Institute. *Base SAS 9.1.3 Procedures Guide.* Cary, NC: SAS Institute, 2006.
37 Hof AL. Scaling gait data to body size. *Gait Posture* 1996; **4**: 222–223.
38 Graham JE, Ostir GV, Kuo YF, Fisher SR, Ottenbacher KJ. Relationship between test methodology and mean velocity in timed walk tests: a review. *Arch Phys Med Rehabil* 2008; **89**: 865–872.
39 Liu MQ, Anderson FC, Schwartz MH, Delp SL. Muscle contributions to support and progression over a range of walking speeds. *J Biomech* 2008; **41**: 3243–3252.
40 Goldberg EJ, Neptune RR. Compensatory strategies during normal walking in response to muscle weakness and increased hip joint stiffness. *Gait Posture* 2007; **25**: 360–367.

41 Fleg JL, Morrell CH, Bos AG *et al.* Accelerated longitudinal decline of aerobic capacity in healthy older adults. *Circulation* 2005; **112**: 674–682.
42 Dobkin BH. Short-distance walking speed and timed walking distance: redundant measures for clinical trials? *Neurology* 2006; **66**: 584–586.
43 Kozakai R, Doyo W, Tsuzuku S *et al.* Relationships of muscle strength and power with leisure-time physical activity and adolescent exercise in middle-aged and elderly Japanese women. *Geriatr Gerontol Int* 2005; **5**: 182–188.
44 Oh-Park M, Zohman LR, Abraham C. A simple walk test to guide exercise programming of the elderly. *Am J Phys Med Rehabil* 1997; **76**: 208–212.
45 Morris JN, Hardman AE. Walking to health. *Sports Med* 1997; **23**: 306–332.
46 Gabell BA, Nayak US. The effect of age on variability in gait. *J Gerontol* 1984; **39**: 662–666.

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Proteolytic processing regulates pathological accumulation in dentatorubral-pallidoluysian atrophy

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Dentatorubral-pallidoluysian atrophy is caused by polyglutamine (polyQ) expansion in atrophin-1 (ATN1). Recent studies have shown that nuclear accumulation of ATN1 and cleaved fragments with expanded polyQ is the pathological process underlying neurodegeneration in dentatorubral-pallidoluysian atrophy. However, the mechanism underlying the proteolytic processing of ATN1 remains unclear. In the present study, we examined the proteolytic processing of ATN1 aiming to understand the mechanisms of ATN1 accumulation with polyQ expansion. Using COS-7 and Neuro2a cells that express the *ATN1* gene, in which ATN1 was accumulated by increasing the number of polyQs, we identified a novel C-terminal fragment containing a polyQ tract. The mutant C-terminal fragment with expanded polyQ selectively accumulated in the cells, and this was also demonstrated in the brain tissues of patients with dentatorubral-pallidoluysian atrophy. Immunocytochemical and biochemical studies revealed that full-length ATN1 and C-terminal fragments displayed individual localization. The mutant C-terminal fragment was preferentially found in the cytoplasmic membrane/organelle and insoluble fractions. Accordingly, it is assumed that the proteolytic processing of ATN1 regulates the localization of C-terminal fragments. Accumulation of the C-terminal fragment was enhanced by inhibition of caspases in the cytoplasm of COS-7 cells. Collectively, these results suggest that the C-terminal fragment plays a principal role in the pathological accumulation of ATN1 in dentatorubral-pallidoluysian atrophy.

Introduction

The polyglutamine (polyQ) diseases are a group of hereditary neurodegenerative disorders that include Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), spinal and bulbar muscular atrophy, and several forms of spinocerebellar ataxia [1–3]. These diseases are caused by expansion of CAG trinucleotide repeats that encode a polyQ tract in the responsible genes. Aside from the CAG trinucleotide repeat, the genes responsible for the various polyQ diseases have no homology to one other. Therefore, speculation concerning the pathogenesis has been focused on the expanded polyQ itself, which appears to cause the gene products to undergo a conformational change that makes them aggregate in neurones [4]. This

Abbreviations
ALLN, *N*-acetyl-Leu-Leu-norleucinal; ATN1, atrophin-1; DRPLA, dentatorubral-pallidoluysian atrophy; GFP, green fluorescent protein; HD, Huntington's disease; HRP, horseradish peroxidase; NLS, nuclear localizing signal; polyQ, polyglutamine; TPEN, *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling; Z-VAD-FMK, benzyloxycarbonyl-Val-Ala-Asp(OMe)-fluoromethyl ketone.

finding suggests that the mechanism of pathogenesis is derived from aggregation of proteins or peptides with the expanded polyQ. By contrast, the onset of a neurological phenotype or cell dysfunction mediated by the expanded polyQ in the responsible gene product was independent of the formation of inclusions [5–7]. Indeed, a previous study showed that the presence of inclusion bodies reduced the risk of neuronal death as a result of polyQ expansion [8]. Thus, the relationship between inclusions and neurotoxicity remains controversial [9]. The polyQ diseases show progressive and refractory neurological symptoms that are caused by neuronal cell loss in selective regions of the central nervous system. This selective neuronal damage gives rise to the specific features of each disease. Accordingly, we hypothesized that each polyQ disease has a distinct molecular mechanism underlying its characteristic neurodegeneration.

DRPLA is an autosomal dominant neurodegenerative disorder characterized clinically by progressive dementia, epilepsy, gait disturbance and involuntary movement (chorea and myoclonus) and, pathologically, by combined degeneration of the dentatorubral and pallidoluysian systems [10,11]. DRPLA pedigrees show genetic anticipation and phenotypic heterogeneity [12–14]. DRPLA is caused by expansion of the polyQ tract within DRPLA protein, also known as atrophin-1 (ATN1). ATN1 is ubiquitously expressed in the central nervous system, although selective regions of the central nervous system are involved in the neuronal degeneration in DRPLA [15]. A previous study using cultured cells expressing ATN1 showed that truncated ATN1 with an expanded polyQ formed perinuclear and intranuclear aggregates and caused apoptotic cell death [16]. Cleavage of ATN1 may be relevant to the disease pathogenesis, although the nature of the relevant cleavage product is uncertain. Previous studies in a transgenic mice model and DRPLA patients have shown that a 120 kDa N-terminal fragment of mutant ATN1 accumulates within the nuclei of neurones [17,18]. On the other hand, we have previously reported evidence of an ~ 100 kDa C-terminal fragment in the normal control and DRPLA human brains [15]. Caspase cleavage of ATN1 at Asp109 generates a large C-terminal fragment [19–21], although whether the caspase cleavage occurs *in vivo* remains uncertain. In the present study, we report a novel C-terminal fragment of ATN1 that contains a polyQ tract found in cellular models of DRPLA, which expresses ATN1 and manifests accumulation of ATN1 with the expanded polyQ. Moreover, the novel C-terminal fragment with the expanded polyQ was discovered in the brain tissues of DRPLA patients. From these results, we hypothesize that pathological ATN1 accumulation underlies neurodegeneration in DRPLA.

Results

Construction of shortened and expanded CAG repeat of *ATN1* gene

The *ATN1* gene was fused to a His-tag and a T7-tag at the 5′-end, and to a *Strep*-tag II at the 3′-end (Fig. 1A). To produce mutant proteins with various numbers of glutamine repeats, we established a method for making the intended CAG repeat a stable PCR product. PCR was performed using oligonucleotides, 5′-$(CAG)_{10}$-3′ and its complementary strand, without DNA templates. The approximately required size of the CAG repeat was obtained by PCR with CAG/CTG oligomer (Fig. S1). The full-length mutant *ATN1* genes were prepared by cassette mutagenesis. The full-length cDNAs of ATN1 with different numbers of the CAG repeat were constructed; the numbers of the translated glutamine repeat are 0, 4, 19, 31, 47, 54 and 77 (Fig. 1B). The polyQ repeat size 0 is a deletion, 4 is shortened, 19 and 31 are normal, 47 is borderline, and 54 and 77 are in the abnormal range. Each expressed protein was represented by adding the number of glutamine repeats it includes after ATN1 (e.g. ATN1-Q19).

Expression of ATN1 in mammalian cells

The cloned cDNA of ATN1 encoded a 1190 amino acid protein that contains the normal 19 polyQ repeat (ATN1-Q19). ATN1 expression systems were constructed for COS-7 and Neuro2a cells. COS-7 and Neuro2a cells were transiently transfected with *ATN1-Q19*-pcDNA3.1 by lipofection. We detected cellular expression of ATN1s with ATN1 antibodies: L55-2 and C580R. Immunoblots of ATN1-Q19 expressed in COS-7 and Neuro2a cells revealed that the ATN1 antibodies labelled two C-terminal fragments of ATN1 with estimated molecular masses of 140 kDa (F1) and 125 kDa (F2), in addition to the full-length ATN1 (Figs 1C,D and S2). The T7-tag antibody detected only the full-length ATN1 at 165 kDa but no fragment (Fig. 1C). Immunoblots of ATN1-Q77 in COS-7 and Neuro2a cells also revealed that L55-2 and C580R recognized the full-length ATN1 at 185 kDa and two C-terminal fragments (Fig. 1C,D). These 160 and 145 kDa fragments corresponded with the mutant F1 fragment with expanded polyQ (mF1) and the mutant F2 fragment (mF2), respectively. The immunoblots of ATN1-Q19 and -Q77 also showed that an antibody

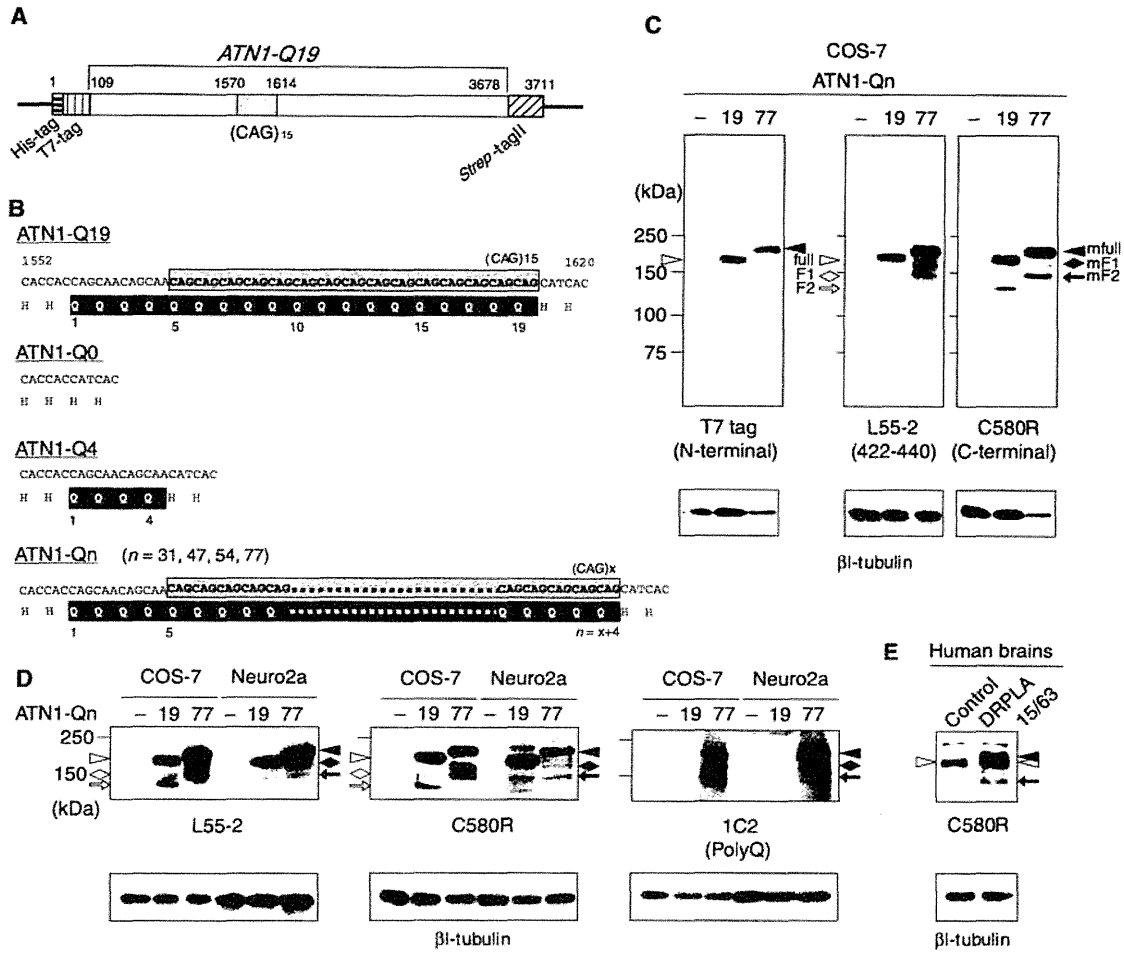

Fig. 1. (A) cDNA constructs of *ATN1* gene and expression of ATN1 in mammalian cells. The ORF of *ATN1-Q19* is shown in the box. The regions encoding the three tags are hatched and the CAG repeat is shown in grey. Numbers above the box represent the positions of the nucleotide counted from the initiation of the cDNA construct. (B) A series of polyQ regions of mutated ATN1 are illustrated. The nucleotides and their corresponding amino acid sequences around the CAG repeat are shown. The regions of CAG repeat in cDNA are shown in grey and the polyQ in the amino acid sequences is shown in black. (C) ATN1-Q19 and -Q77 were expressed in COS-7 cells. Expressed ATN1 was detected by immunoblotting using T7-tag, L55-2 and C580R antibodies. The immunoblots revealed that the full-length ATN1 was cleaved into two fragments containing the C-terminal and polyQ tract. The arrowheads show the full-length ATN1-Q19 (white) and full-length ATN1-Q77 (black). C-terminal fragments are defined as F1 (white lozenge) and F2 (white arrow). In ATN1-Q77, they are defined as mF1 (black lozenge) and mF2 (black arrow). βI-tubulin was examined as a loading control. (D) We expressed ATN1-Q19 and -Q77 in COS-7 and Neuro2a cells. ATN1s expression was compared using immunoblotting with ATN1 antibodies (C580R and L55-2) and polyQ antibody (1C2). The antibodies showed no difference in immunoreactivity of ATN1 and various fragments between the Neuro2a and COS-7 cells. 1C2 labelled the ATN1-Q77 bands but not the ATN1-Q19 band. βI-Tubulin was used as a loading control. Representative immunoblots of three independent experiments are shown. (E) Tissue samples of the cerebellum of a patient with DRPLA and the human control brain tissue were examined by immunoblotting. The antibody C580R recognized the C-terminal of ATN1 mutant (black arrowhead) and wild-type (white arrowhead), the full-length ATN1s in the DRPLA brain tissue. A novel C-terminal fragment mF2 with an expanded polyQ tract (black arrow) was identified in the DRPLA brain tissue.

against polyQ tracts, 1C2, detected the same immunoreactivity of ATN1 and fragments as L55-2 (Fig. 1D), which indicates that the C-terminal fragments of mF1 and mF2 contained polyQ tracts.

Furthermore, the brain tissues from DRPLA patients also contained the C-terminal fragment of ATN1 containing an expanded polyQ tract. Immunoblots of the brain tissues from DRPLA patients revealed an immunoreactive, C580R-labelled band at ~ 150 kDa, which corresponds with the results of mF2 fragment of ATN1-Q77 in COS-7 cells (Fig. 1E, black arrow). Taken together, these results demonstrated that the mutant, full-length ATN1 was cleaved into the C-terminal fragment of mF2 in the mammalian cultured cells and