表 2　二次性サルコペニア・モデル

	操作後の評価期間	関連文献
廃用，神経障害など		
denervation(坐骨神経切除)	7 日	Hishiya, A. ら[15]
hind-limb suspension(後肢懸垂モデル)	14 日	Haida, N. ら[16]
hindlimb immobilization(後肢固定)	7 日	Caron, A. Z. ら[17]
cachexia(炎症，悪性腫瘍，臓器不全など)		
collagen-induced arthritis	35 日	Filippin, LI. ら[18]
敗血症		
lipopolysaccharide	18 時間後(筋肉重量，蛋白量)	Jin, B. and Li, Y. P.[19]
細菌(S. aureus)	24 時間後(筋肉蛋白分解)	Khal, J. and Tisdale, M. J.[20]
悪性腫瘍		
Lewis lung carcinoma	15 日	Busquets, S. ら[21]
Murine adenocarcinoma 16	18 日	Cannon, T. ら[22]
C26 colorectal adenocarcinoma	25 日	Zhou, X. ら[23]
臓器不全		
腎不全(腎部分摘除)	14 日	Cheung, W. ら[24]
心不全(冠動脈結紮)	6 週	Yoshida, T. ら[25]
低栄養		
2 日間の水分のみ	2 日	Kudryashova, E. ら[26]

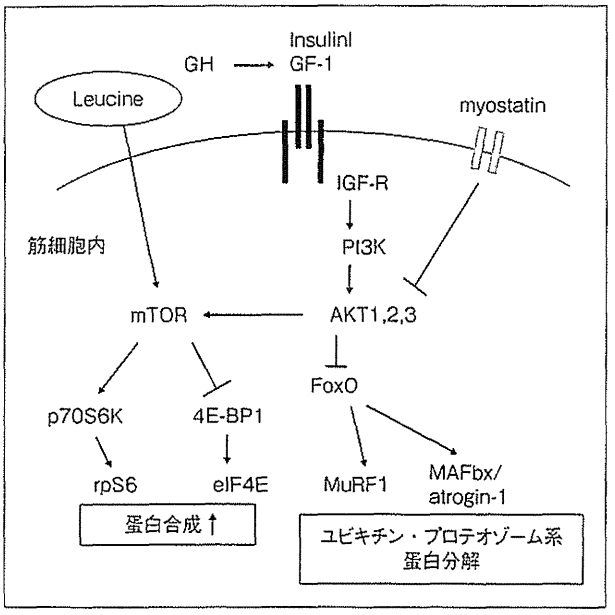

図 1　筋肉蛋白質の合成，分解にかかわるシグナル

GH：growth hormone, IGF-Ⅰ：insulin-like growth factor 1, IGF-R：insulin-like growth factor receptor, PI3K：phosphoinositide 3-kinase, AKT：protein kinase B(PKB), FOXO：Forkhead box O, mTOR：mammalian target of rapamycin, MuRF1：Muscle RING-Finger Protein-1, 4E-BP1：eukaryotic initiation factor 4-binding protein 1, eIF4E：Eukaryotic Initiation factor 4E.

る．餌の摂取はできるようにするが，2 週間の観察中に体重減少が進む．したがって，この期間の後肢の骨格筋萎縮は顕著ではあるが，廃用だけによるものかは疑問が残る．これ以外のも閉塞性肺疾患や肝不全モデルでも骨格筋萎縮は誘導される．

遺伝子操作マウス[27-37]

筋肉の肥大ならびに萎縮は，筋肉内での筋蛋白質の合成分解のバランスが崩れることにより誘導される．図 1 は代表的な筋肉蛋白質合成ならびに分解にかかわるシグナルを模式化したものである．筋肉蛋白合成系は筋肉蛋白合成・分解にかかわるシグナルの操作により筋肉の萎縮が誘導され，サルコペニアのモデルまたはサルコペニアに関与する分子が同定できる可能性がある．

Growth hormone(GH)，insulin-like growth

表 3　遺伝子操作マウスとサルコペニア

ターゲット遺伝子	発現	body size	muscle size	muscle performance	life span	関連文献
Akt1	欠損	↓	↓(とくに type Ⅱ fiber)		↓	Goncalves, M. D. ら[28]
Akt2	欠損	→	↓			
Akt1+2	欠損	↓	↓			Peng, X. D. ら[29]
Akt	高発現	↑	↑	↑		Rommel, C. ら[30]
mTOR(muscle)	欠損	↓	↓		↓	Risson, V. ら[31]
mTOR	発現低下	↓		↑	↑	Wu, J. J. ら[32]
FoxO1	高発現	↓	↓	↓		Kamei, Y. ら[33]
FoxO1	欠損		↓(とくに type Ⅰ fiber)			Kitamura, T. ら[34]
MuRF1	欠損	→	→(denervation後の萎縮は抑制)			Bodine, S. C. ら[35]
MAFbx	欠損	→	→(denervation後の萎縮は抑制)			
MuRF1	欠損	→	→		→	Witt, C. C. ら[36]
MuRP2	欠損	→	→		→	
MuRF1+2	欠損	↓	↑		↓	
myostatin	欠損	↑	↑		→	Morissette, M. R. ら[37]

Akt：protein kinase B(PKB), mTOR：mammalian target of rapamycin, FoxO1：Forkhead box O1, MuRF：Muscle RING-Finger Protein.

factor-1(IGF-Ⅰ)受容体の遺伝子操作により，この経路が十分機能しない場合，成長が遅延し，骨格筋の萎縮を認めることが知られる[27]．しかし，この遺伝子操作モデルは骨格筋を含め正常な成長が起こらず，筋発育不全のモデルにはなりうるが，サルコペニアのモデルとはいえない．

そのほか，図1にある筋肉内の蛋白合成，分解にかかわる種々のシグナルへの遺伝子操作により骨格筋の肥大，萎縮は表3のように報告されている．しかし，これらはけっして加齢によるサルコペニアのモデルではなく，そのメカニズムを解明する際に有用である．

おわりに

サルコペニアのメカニズムの解明や治療法の開発に使用できる可能性のあるマウスモデルを提示した．二次性のサルコペニアのモデルは，本稿であげたもの以外にそのほか多数のモデルが存在する．その用途に合わせて使用していただきたい．原発性サルコペニアの研究にあたっては野生型を使用するのが基本であろうが，高齢マウス・コロニーから手に入れることができればいいが，自施設で一から作成しようと思うとたいへん時間がかかる．その意味では，老化促進マウスは使用しやすい．しかし，老化以外の要因が関与するリスクはある．

文献

1) Rosenberg, I. H.：*Am. J. Clin. Nutr.*, **50**：1231-1233, 1989.
2) Cruz-Jentoft, A. J. et al.：*Age Ageing*, **39**：412-423, 2010.
3) Andersen, J. L.：*Scand. J. Med. Sci. Sports*, **13**：40-47, 2003.
4) Kanda, K. and Hashizume, K.：*J. Neurophysiol.*, **61**：737-746, 1989.
5) Mehl, K. A. et al.：*J. Appl. Physiol.*, **99**：2379-2387, 2005.
6) Lexell, J.：*J. Gerontol A Biol. Sci. Med. Sci.*, **50**：11-16, 1995.
7) Romanick, M. et al.：*Biochim. Biophys. Acta*, **1832**：1410-1420, 2013.
8) Sheard, P. W. and Anderson, R. D.：*Biogerontology*, **13**：157-167, 2012.
9) Derave, W. et al.：*Exp. Gerontol.*, **40**：562-572, 2005.
10) 藤田慎一・他：久留米大学保健体育センター研究機構，**5**：1-5，1997.
11) Iida, R. H. et al.：*Mol. Cell. Biochem.*, **348**：89-98, 2011.
12) Hiona, A. et al.：*PLoS One*, **5**：e11468, 2010.
13) Greising, S. M. et al.：*Age(Dordr)*, **34**：805-819, 2012.
14) Didier, N. et al.：*EMBO Mol. Med.*, **4**：910-923, 2012.
15) Hishiya, A. et al.：*EMBO J.*, **25**：554-564, 2006.
16) Haida, N. et al.：*Exp. Neurol.*, **103**：68-76, 1989.
17) Caron, A. Z. et al.：*J. Appl. Physiol.*, **106**：2049-2059, 2009.
18) Filippin, L. I. et al.：*J. Cachexia Sarcopenia Muscle*, **4**：231-238, 2013.

19) Jin, B. and Li, Y. P. : *J. Cell Biochem.*, **100** : 960-969, 2007.
20) Khal, J. and Tisdale, M. J. : *Biochem. Biophys. Res. Commun.*, **375** : 238-240, 2008.
21) Busquets, S. et al. : *Cancer Res.*, **64** : 6725-6731, 2004.
22) Cannon, T. et al. : *Laryngoscope*, **117** : 2152-2158, 2007.
23) Zhou, X. et al. : *Cell*, **142** : 531-543, 2010.
24) Cheung, W. et al. : *J. Clin. Invest.*, **115** : 1659-1665, 2005.
25) Yoshida, T. et al. : *J. Biol. Chem.*, **288** : 23823-23832, 2013.
26) Kudryashova, E. et al. : *J. Clin. Invest.*, **122** : 1764-1776, 2012.
27) Mavalli, M. D. et al. : *J. Clin. Invest.*, **120** : 4007-4020, 2010.
28) Goncalves, M. D. et al. : *PLoS One*, **5** : e12707, 2010.
29) Peng, X. D. et al. : *Genes Dev.*, **17** : 1352-1365, 2003.
30) Rommel, C. et al. : *Nat. Cell Biol.*, **3** : 1009-1013, 2001.
31) Risson, V. et al. : *J. Cell Biol.*, **187** : 859-874, 2009.
32) Wu, J. J. et al. : *Cell Rep.*, **4** : 913-920, 2013.
33) Kamei, Y. et al. : *J. Biol. Chem.*, **279** : 41114-41123, 2004.
34) Kitamura, T. et al. : *J. Clin. Invest.*, **117** : 2477-2485, 2007.
35) Bodine, S. C. et al. : *Science*, **294** : 1704-1708, 2001.
36) Witt, C. C. et al. : *EMBO J.*, **27** : 350-360, 2008.
37) Morissette, M. R. et al. : *Aging Cell*, **8** : 573-583, 2009.

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特集Ⅰ 健康長寿のためのシニアニュートリション

Prevention of Sarcopenia and the Appropriate Nutrition

サルコペニア予防と栄養

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はじめに

サルコペニアは「加齢に伴う筋力の低下、または老化に伴う筋肉量の減少」を指し、Rosenberg IHにより提唱された比較的新しい造語である[1]。一般的に70歳までに20歳台と比較すると骨格筋面積は25～30%、筋力は30～40%減少し、50歳以降毎年1～2%程度筋肉量は減少すると言われている[2]。サルコペニアの存在は、高齢者では「ふらつき」、「転倒」、さらには「虚弱（フレイルティ）」に密接に関連し、その先には要介護状態が待ち受けている[2]。従ってサルコペニアの原因を究明し、それに沿った介入法を開発、導入することは介護予防の観点からも超高齢社会に突入した我が国においては、医療・介護政策上の観点からも極めて重要である。

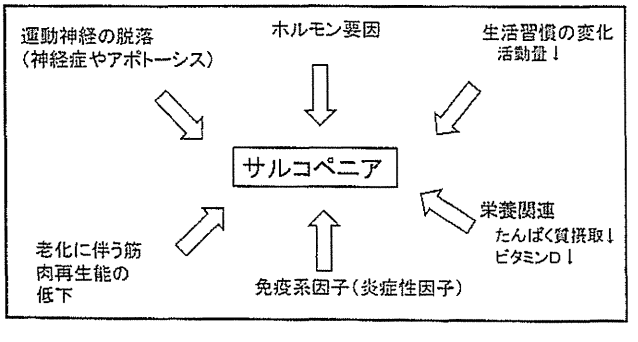
1. サルコペニアのメカニズム、特に栄養との関連

(1) サルコペニアの要因

加齢とともに骨格筋は筋線維数の減少だけではなく、一つ一つの筋線維自体も萎縮する。主に減少する筋線維はタイプⅡ筋線維で、速筋と言われるものである。しかし、最近ではタイプⅡだけではなく、80歳を超えるとタイプⅠ筋線維も同様に減少してくるとする報告も多い。興味深いことに四肢骨格筋の加齢に伴う減少は上肢よりも下肢でより著しいと報告されている[3]。

筋肉自体の減少に伴い、脂肪や細胞間質が増加する。実際CTやMRI検査では筋肉組織の減少に伴い、脂肪や細胞外線維などが筋肉間に浸潤しているのが観察される。従って、DXA法（二重エネルギーエックス線吸収測定法）などで計測される四肢骨格筋量よりも実際の骨格筋量はより減少していることが多い。筋線維を支配している運動神経細胞（運動ニューロン）は脊髄にあって、ここから出た神経線維は幾重にも分枝して筋線維に到達する。運動ニューロンとそれが支配している筋線維をまとめて運動単位というが、加齢とともに、この運動単位が減少することが知られている[4]。また骨格筋再生に重要で骨格筋細胞周囲に存在する筋芽細胞に分化する衛星細胞自体の数も減少と報告され、さらには加齢により筋衛星細胞の筋芽細胞への分化が抑制されているとの報告が多い[5]。筋肉細胞自体の萎縮は筋たんぱく質の減少を伴っており、筋肉たんぱくの同化・異化バランスがこの病態に関わっている可能性が高い。このようにサルコペニアは多因子が関わっている病態である（図1）。

図1 様々なサルコペニアの要因

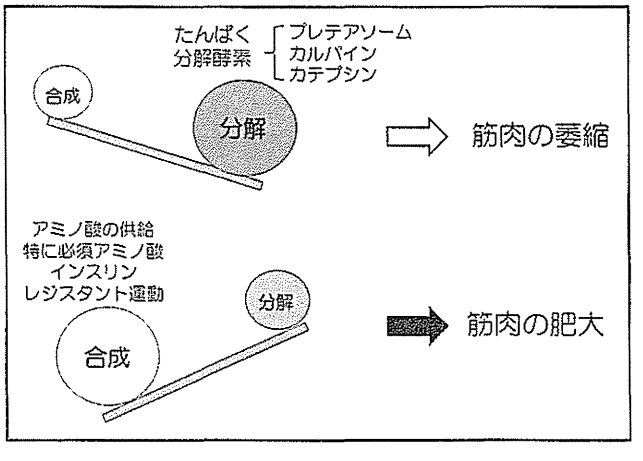


(2) たんぱく質とサルコペニア

筋肉の肥大、萎縮は単純に言うと筋肉たんぱく質の合成（同化）と分解のバランスで決まる（図2）。たんぱく合成は図2や以下に述べるような様々刺激で誘導され、また分解は筋肉細胞にある様々な分解システムにより促進される。筋肉たんぱくは様々な状況下で分解するため、筋肉量を維持するためには筋細胞内でのたんぱく合成が必須である。筋肉たんぱくの合成にはその原料となるアミノ酸が必須であり、さらにその上流にあるたんぱく質の摂取が必須である。

加齢とともに摂取したたんぱく質が効率的に吸収されないのではないか、との報告も以前は認められたが、現在では少なくとも多くの健康な高齢者では若年者と同様に摂取されたたんぱく質は消化管で分解、吸収され加齢の影響は疾病を合併していない限りあまりないことが報告されている。また、加齢と伴に筋肉でのたんぱく合成能が低下するのではないかとの見解もあり、実際、アミノ酸摂取に対しての筋肉のたんぱく同化反応の感受性が低下しているとの報

図2 筋肉たんぱく質の合成と分解のバランス



告がある[6]。

摂取たんぱく質を増やすことだけにより筋肉量が増加するかどうかは議論があるところだが、減少を予防することはできる可能性がある。実際、地域高齢者の観察研究（the Health, Aging, and Body Composition（Health ABC）Study）では摂取カロリー当たりのたんぱく質量により3年後の除脂肪体重ならびに四肢除脂肪体重の低下率が変化し、たんぱく質摂取が多いほどその低下率が低いことが報告された[7]。

一方高たんぱく質の摂取による体への悪影響（腎毒性など）が指摘されているが、腎機能の低下がない場合には極端な高たんぱく食でない限り、重大な副作用につながることはまれである。実際筋肉量の減少をきたしやすい高齢者が筋肉量を維持するには、1.0～1.3g/kg/day程度の摂取が必要との指摘もある。欧米からの報告では1.6g/kg/dayのたんぱくで運動による筋肉量増加を認めたとか、1.0g/kg/dayが筋肉量の低下を予防する最低限のタンパク質摂取量だなどの報告もある[8,9]。しかし、高齢者では腎機能が低下していることも少なくないため、1.5g/kg/day以上を摂取させる際には十分注意を要する。現在日本人の食事摂取基準では高齢者でも成人と同様男性で推定平均必要量を50g/day、推奨量を60g/day、女性で推定平均必要量を40g/day、推奨量を50g/dayとしているが、今後この量でサルコペニア予防が実現できるかの検証が必要である[10]。

(3)アミノ酸とサルコペニア

正常な筋肉たんぱく質代謝のためにはアミノ酸の筋肉への供給が不可欠である。アミノ酸には体内で合成できるか、できないかにより非必須アミノ酸と必須アミノ酸に分けるが、筋肉のたんぱく同化作用は主に必須アミノ酸に依存していることが知られる。筋肉を構成しているアミノ酸のうち30～40%が必須アミノ酸であるとも言われている。必須アミノ酸がなぜ筋肉においてたんぱく同化として機能するかは十分解明されていないが、必須アミノ酸の供給は単にたんぱく質合成の原料として使用されるだけではなく筋肉細胞に直接働いてたんぱく質合成を刺激している。必須アミノ酸の中でも分枝鎖アミノ酸（ロイシン、イソロイシン、バリン）、さらにはその中でもロイシンは筋たんぱく合成刺激が強いことが知られる（図3）[11]。ロイシンは70-kDa ribosomal protein S6 kinase（p70S6K）やeukaryotic initiation factor 4E binding protein-1（4E-BP1）を含むthe mammalian target of rapamycin（mTOR）pathwayを介してたんぱく同化作用を示すことが知られている(図3)。

一方、高齢者では若年者と比較しロイシンのたんぱく同化作用が低下しているとの報告がある。その機構としては高齢者での骨格筋では若年者と比較しmTORならびにその下流のS6Kの経路の活性化が低下していると言われている。しかし、十分量なロイシンに対してはたんぱく同化作用は健在で筋肉たんぱく質の合成に傾く。このことは高齢者の骨格筋ではロイシンが低濃度だとそのたんぱく質同化の刺激が弱く、たんぱく合成に働かない可能性がある。しかし、十分量のロイシンが加えられれば若年者と同様にたんぱく合成が増加することを意味する。

2. 運動と栄養

栄養の補給だけでは十分に骨格筋の増強作用は不十分であることが指摘され、運動との併用が効果的と報告されている[12]。一方、運動、特にレジスタンス運動のサルコペニアに対する効果がいくつか報告されている。しかし、運動だけでも効果は少ないと言われている。実際、空腹時での運動では筋肉でのたんぱく合成は誘導されるが、同時に分解も促進されることが報告されており、十分なたんぱく質の供給がレジスタンス運動にも必要である[13]。上でも述べたが、報告によると、高齢者で運動とともに1.6g/kg/dayのたんぱく質摂取で筋肉量の増大を認め、最低限1.0g/kg/dayのたんぱく質摂取が必要とされている。

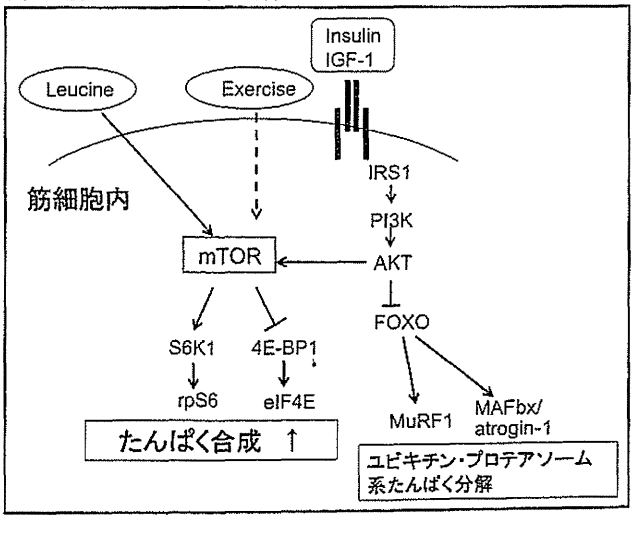


図3　筋たんぱく合成・分解経路

3. ビタミンD

高齢者ではビタミンD欠乏に陥りやすい。ビタミンD血中濃度とサルコペニアとの関連は横断的のみならず縦断的研究でも報告されている。例えば65歳以上の高齢者で25-hydroxyvitamin Dの値と3年後のサルコペニアの存在との関係を検討すると、ビタミンDが低値であるとサルコペニアのリスクが増加する[14]。ビタミンDは1,25（OH）D核内受容体を介してカルシウム・リン輸送、リン脂質代謝、筋細胞の増殖、分化に影響を与えることが知られている。一方ビタミンDによる筋力の増強、転倒予防に関する介入試験の結果は必ずしも一致していない[15]。しかし最近のビタミンD低値の高齢者を対象とした介入試験では筋力、転倒に対して良好な効果が報告されている[16]。今後さらなるデータの蓄積が期待される。

おわりに

冒頭で述べたようにサルコペニアの要因は多義にわたり、老化に伴う要因はおそらく一つの要因と言うよりも多因子が複雑に関連し合って筋肉萎縮に至るものと想像される。従って、十分な栄養だけ摂取すればサルコペニアが完全に予防されるわけではない。しかし、栄養不足（欠乏）がサルコペニアの危険因子、促進因子になっていることは明らかであり、必要十分な栄養を摂取することは高齢者にとって重要である。その他の栄養素、例えば抗酸化ビタミン、ビタミンB_6、B_{12}、葉酸のようなホモシステイン関連ビタミン、また脂肪酸などとサル

コペニアとの関連を示唆する報告もあるが、十分な証拠があるとは今のところ言えなく、今後のデータの蓄積が必要である。

〈参考文献〉

1) I.H. Rosenberg. Summary comments. Am J Clin Nutr. 50, 1231-3(1989).
2) 葛谷雅文.老年医学におけるSarcopenia &Frailtyの重要性.日老医誌 46: 279-285, (2009)
3) I. Janssen, S.B. Heymsfield, Z.M. Wang, *et al.* Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. J Appl Physiol, 89, 81-8 (2000)
4) J. Lexell, K. Henriksson-Larsen, B. Winblad, *et al.* Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. Muscle Nerve, 6, 588-95 (1983).
5) A. Bigot, V. Jacquemin, F. Debacq-Chainiaux, *et al.* Replicative aging down regulates the myogenic regulatory factors in human myoblasts. Biol Cell, 100, 189-199 (2008).
6) E. Volpi, B. Mittendorfer, B.B. Rasmussen, *et al.* The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. J Clin Endocrinol Metab. 85, 4481-4490 (2000).
7) D.K. Housto, B.J.Nicklas, J.Ding, *et al*: Health ABC Study. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 87, 150-155,(2008).
8) W.W.Campbell, T.A.Trappe, R.R.Wolfe, W.J.Evans. The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. J Gerontol A Biol Sci Med Sci. 56, M373-80 (2001).
9) W.W.Campbel, C.A.Johnson, G.P.McCabe, N.S.Carnell. Dietary protein requirements of younger and older adults. Am J Clin Nutr. 88,1322-9 (2008).
10) 日本人の食事摂取基準(2010年度版).「日本人の食事摂取基準」策定検討会報告書 平成21年5月 厚生労働省
11) M.H.Stipanuk. Leucine and protein synthesis: mTOR and beyond. Nutr Rev. 65:122-129, (2007).
12) M.A.Fiatarone, E.F.O'Neill, N.D.Ryan ND, *et al.* Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 330: 1769-1775, (1994).
13) G.Biolo, K.D.Tipton, S.Klein, *et al.* An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol. 273 (1 Pt 1),E122-129, (1997).
14) M.Visser, D.J.Deeg, P.Lips. Longitudinal Aging Study Amsterdam. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab. 88,5766-5772, (2003).
15) K.A.Stockton, K.Mengersen, J.D.Paratz, *et al.* Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. Osteoporos Int. 22, 859-871, (2011).
16) K.Zhu , N.Austin , A.Devine, *et al.* A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. J Am Geriatr Soc. 58, 2063-2068, (2010).

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ORIGINAL ARTICLE

Association between hypertension status and the screening test for frailty in elderly community-dwelling Japanese

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To clarify the possible association of frailty with hypertension prevalence, treatment and blood pressure (BP) control in the elderly, we conducted a screening survey of 1091 elderly community-dwelling subjects aged ⩾65 years, using data from public health check-ups and frailty was determined by a 25-item questionnaire, the Basic Checklist for Frailty (BCF). The significance of differences in the association of BCF categories or BCF items with each hypertension status was analyzed using multiple logistic regression analysis after adjusting for age, sex and possible confounding underlying chronic conditions. A total of 63% of subjects were hypertensive (BP⩾140/90 mm Hg), and of those, 85% were receiving antihypertensive treatment, and 56.0% of those receiving treatment had controlled BP (<140/90 mm Hg). BCF categories that showed an independent association with hypertension status were 'impaired walking status' and absence of 'impaired nutritional status' for prevalence of hypertension, 'impaired instrumental activity of daily living status' and 'impaired nutritional status' for untreated hypertension among hypertensives and 'impaired oral function' for BP-uncontrolled hypertension among treated hypertensives. In addition, BCF items that showed an independent association were 'inability to walk for more than 15 min without rest' and absence of 'Body mass index (BMI) <18.5 kg m^{-2}' for prevalence of hypertension, 'weight loss of more than 2–3 kg in the past 6 months' for untreated hypertension, and 'difficulty eating hard food' for BP-uncontrolled hypertension. These observations indicate that assessment of these specified frailty categories and/or items may be useful for evaluating hypertension status in elderly community-dwelling subjects.
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INTRODUCTION

Providing high quality care to older adults with hypertension is growing in importance because of improved survival of patients with hypertension into old age and a growing older population at risk of developing hypertension.[1] Many large-scale intervention trials have proven the necessity of treatment of hypertension in the elderly, including isolated systolic hypertension. Meta-analyses of large-scale intervention trials for elderly hypertensive patients aged 60 years and older,[2] as well as for those aged 80 years and older,[3] have revealed significant reductions in morbidity and/or mortality of cerebro-cardiovascular disease by antihypertensive treatment. Moreover, the Hypertension in the Very Elderly Trial directly and clearly revealed a beneficial effect of antihypertensive treatment even in those aged 80 years and older.[4]

Trends in hypertension prevalence, treatment and control over time have been reported in US adults,[5] including those aged 60 and older[6] using data from two independent national surveys: the National Health and Nutrition Evaluation Survey (NHANES) III (1988–1994) and the current NHANES (1999–2004). The older population with hypertension has been reported to have poorer blood pressure (BP) control than younger populations in the US.[7,8] The prevalence of hypertension in community-dwelling Japanese has also been reported to increase with age from 20 years through 80 years, reaching 50% and higher at 75 years of age and older in both sexes.[9] However, little is reported about trends in the treatment and BP-control of hypertension in elderly community-dwelling subjects in Japan.

On the other hand, hypertension is also known to be linked with frailty in the elderly, as assessed by weight loss, low activities of daily living (ADL), low instrumental ADL (IADL) and low physical activity.[10,11] In Japan, the public long-term care insurance system provides services to older adults who have been certified as requiring support (levels 1–2) or care (levels 1–5). Uncertified older adults with impaired health who are considered at high risk for needing support/care (frail elderly) are provided with preventive care services by municipalities.[12] Uncertified elderly subjects are given an annual health check-up by the local government, and frailty is examined using the Basic Checklist for Frailty (BCF), a yes-no questionnaire

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consisting of simple assessments for seven categories of frailty; impaired IADL status (five items), impaired walking status (five items), impaired nutritional status (two items), impaired oral function (three items), staying indoors (two items), impaired memory status (three items) and depressed mood (five items). However, few studies have examined the association of hypertension prevalence, treatment and BP-control with frailty in the elderly. Therefore, this study examined the relationship between hypertension status, and BCF categories or items in elderly community-dwelling subjects. We also studied whether this relationship could be explained by underlying chronic conditions.

METHODS

Subjects

In April 2008, the Regional Comprehensive Support Center of Uchinada-Town, Ishikawa, Japan distributed the BCF to all uncertified elderly community-dwelling subjects aged $\geqslant$65 years. The local government also provided a Public Health Center-based annual health check-up to these elderly subjects. Data were collected by the Uchinada-Town local government after depersonalizing participant data to ensure anonymity. We excluded elderly subjects who were already certified for long-term care insurance at the baseline. The study was formally approved by the Clinical Research Ethics Committee of Kanazawa Medical University.

Baseline examinations

A self-administered questionnaire that included medical history, smoking condition (yes/no), regular alcohol drinking (yes/no) and time since the last meal[13] was completed at baseline. BMI, $kg\,m^{-2}$ was calculated as weight divided by height squared. The blood condition was defined as fasting if blood was collected more than 8 h after the last meal. Serum levels of Cr, total cholesterol, HDL-cholesterol, triglycerides and glucose were measured using an automated spectrometer. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate, calculated by the Modification of Diet in Renal Disease equation[14] with coefficients modified for Japanese patients,[15] $194 \times Cr^{-1.094} \times age^{-0.287}$ ($\times 0.739$ if female), $<60\,ml\,min^{-1}\ 1.73\,m^{-2}$. Diabetes mellitus was defined as a fasting blood glucose $\geqslant 7.0\,mmol\,l^{-1}$ ($126\,mg\,dl^{-1}$), a non-fasting glucose level $\geqslant 11.1\,mmol\,l^{-1}$ ($200\,mg\,dl^{-1}$), HbA1c $\geqslant$6.5% by a standardized method, or use of hypoglycemic agents and/or insulin.[16] Dyslipidemia was defined as fasting plasma total cholesterol level $\geqslant 5.72\,mmol\,l^{-1}$ ($220\,mg\,dl^{-1}$), triglycerides $\geqslant 1.70\,mmol\,l^{-1}$ ($150\,mg\,dl^{-1}$), high-density lipoprotein cholesterol $<1.04\,mmol\,l^{-1}$ ($40\,mg\,dl^{-1}$), or use of lipid-lowering agents.[17]

Hypertension status

Baseline BP was measured at least twice from the right arm of seated participants who had rested for more than 5 min, by trained observers using standard mercury sphygmomanometers. When the difference in the two measurements of systolic BP was greater than 5 mm Hg, another measurement was performed.[18] The mean of the last two stable measurements was used for analyses. Hypertension was defined as systolic BP $\geqslant$140 mm Hg, diastolic BP $\geqslant$90 mm Hg, or current antihypertensive drug treatment. Treatment was defined as reported current use of antihypertensive drug therapy. BP-control was defined as antihypertensive drug treatment associated with systolic BP $<$140 and diastolic BP $<$90 mm Hg.

Statistical methods

For comparison of two groups, we used univariate analysis including χ^2 test (Fisher's exact test when needed) for comparing categorical variables and nonparametric Mann–Whitney *U* statistics for comparing the distributions of ordinal variables. Logistic regression analysis was used to identify frailty factors independently associated with any of prevalence of hypertension among all elderly subjects, untreated hypertension among hypertensive subjects and BP-uncontrolled hypertension among treated hypertensive subjects, after adjustment for age, sex and associated variables by univariate analysis. Common pitfalls associated with multivariate regression were avoided as described by Concato *et al.*[19] Associated variables were selected from the data sets of status of smoking and alcohol intake, past history of stroke and ischemic heart disease, presence of CKD, diabetes mellitus and dyslipidemia and either the seven BCF categories (model-1) or the 25 questionnaire items (model-2), according to their univariate analysis *P*-value ($<$0.20) to avoid common pitfalls associated with multivariate regression.[19] Estimates for odds ratio and corresponding two-sided 95% confidence interval demonstrating statistical significance were derived from the regression model. Data were analyzed using SPSS (v. 16.0, Chicago, IL, USA). A probability of $P<0.05$ was taken as statistically significant.

RESULTS

Study population

A primary screening questionnaire survey was conducted on all 4050 uncertified elderly community-dwelling subjects, aged $\geqslant$65 years, living in a town in Ishikawa, Japan. Out of 3150 (77.8%) subjects who replied to the questionnaire, 1091 (427 men and 664 women) supplied complete information on all study variables, including the health check-up and were included in our study. The age of subjects (mean $\pm$ s.d.) was 73.5 $\pm$ 6.1 years (65–94 years). In the 1091 included individuals, the significance of differences in clinical factors was analyzed using univariate (Table 1) and multivariate (Tables 2 and 3) comparisons of hypertensives ($n=683$) and normotensives ($n=408$), untreated ($n=104$) and treated ($n=579$) hypertensives and BP-uncontrolled ($n=255$) and BP-controlled ($n=324$) treated hypertensives (Figure 1). A total of 62.6% of subjects were hypertensive, and of those, 84.8% were receiving antihypertensive drug treatment and BP was controlled in 56.0% of those undergoing treatment. Overall, 47.4% of hypertensive patients had controlled BP.

Baseline factors and BCF categories and items

Compared with non-hypertensive elderly subjects, hypertensive subjects were older, and showed a higher prevalence of concomitant diabetes mellitus and dyslipidemia in univariate analysis (Table 1). Moreover, hypertensive subjects were less active and less thin than non-hypertensive subjects, as shown by associations with 'impaired walking status' and absence of 'impaired nutritional status' in the BCF categories, and associations with six BCF items including one IADL item, four items of 'impaired walking status' and absence of 'BMI $<18.5\,kg\,m^{-2}$' in univariate analysis (Table 1). Multiple logistic analysis using these BCF categories selected by univariate *P*-values $<$0.20 (model-1) revealed that two BCF categories, 'impaired walking status' and absence of 'impaired nutritional status', besides older age and diabetes mellitus, showed statistically significant association with prevalence of hypertension in elderly subjects (Table 2). In multiple logistic analysis using model-2 sets, two BCF items, 'inability to walk for more than 15 min without rest' and absence of 'BMI $<18.5\,kg\,m^{-2}$', besides older age and diabetes mellitus, showed statistically significant association with prevalence of hypertension in elderly subjects (Table 3).

In contrast to the entire hypertensive subjects, untreated hypertensive subjects were associated not only with clinical factors, namely absence of CKD or dyslipidemia and female sex, but also with one BCF category, 'impaired nutritional state' and with eight BCF items, including four out of five IADL items: 'able to stand up', 'weight loss of more than 2–3 kg in the past 6 months', 'going out more than once a week' and 'able to make a phone call', compared with treated hypertensive subjects in univariate analysis (Table 1). Logistic regression analysis using model-1 sets revealed that two BCF categories, 'impaired IADL status' and 'impaired nutritional

Table 1 Characteristics of subjects according to hypertension, drug treatment and control of hypertension in community-dwelling elderly Japanese

	Hypertension			*Treatment*			*Control*		
	No	*Yes*	P	*No*	*Yes*	P	*No*	*Yes*	P
Clinical background	*n*=408	*n*=683		*n*=104	*n*=579		*n*=255	*n*=324	
Age (years)	72.0 (6.0)	74.4 (6.1)	<0.001	75.1 (6.0)	74.3 (6.1)	0.142	73.6 (6.0)	74.8(6.1)	0.015
Sex (% female)	58.3	62.4	0.186	71.2	60.8	0.048	63.9	58.3	0.172
Current smoker (%)	18.6	13.9	0.038	6.7	15.2	0.022	16.8	13.9	0.323
Regular alcohol drinker (%)	25.6	30.6	0.073	21.1	26.4	0.257	26.7	26.2	0.907
Systolic BP (mm Hg)	122 (12)	139(14)	<0.001	148 (10)	137 (14)	<0.001	149 (11)	127 (8)	<0.001
Diastolic BP (mm Hg)	74 (8)	79 (9)	<0.001	84 (8)	78 (9)	<0.001	83 (9)	75 (7)	<0.001
Clinical findings on admission									
Past history of stroke (%)	3.9	5.9	0.154	8.7	5.4	0.204	4.0	6.5	0.198
Ischemic heart disease (%)	1.8	3.4	0.135	2.9	3.5	0.745	4.0	3.1	0.545
CKD (%)	34.8	35.9	0.721	23.1	38.2	0.003	35.3	40.4	0.206
Diabetes mellitus (%)	11.0	20.4	<0.001	13.5	21.6	0.058	19.6	23.1	0.304
Dyslipidemia (%)	21.7	27.3	0.042	10.6	30.4	<0.001	26.7	33.2	0.094
Category of BCF									
Impaired instrumental activity of daily living (five items)	0.58 (1.05)	0.63 (1.10)	0.381	0.97 (1.55)	0.56 (0.99)	0.064	0.63 (1.06)	0.51 (0.93)	0.188
Impaired walking status (five items)	0.81 (1.18)	1.14 (1.36)	<0.001	1.37 (1.57)	1.10 (1.32)	0.179	1.13 (1.33)	1.08 (1.32)	0.741
Impaired nutritional status (two items)	0.22 (0.45)	0.17 (0.40)	0.049	0.28 (0.51)	0.15 (0.38)	0.004	0.14 (0.38)	0.16 (0.38)	0.532
Impaired oral function (three items)	0.57 (0.78)	0.61 (0.83)	0.590	0.67 (0.92)	0.60 (0.82)	0.615	0.67 (0.88)	0.54 (0.76)	0.195
Staying indoors (two items)	0.21 (0.47)	0.24 (0.49)	0.198	0.31 (0.57)	0.27 (0.47)	0.222	0.23 (0.47)	0.23 (0.48)	0.785
Impaired memory status (three items)	0.30 (0.58)	0.36 (0.67)	0.207	0.49 (0.85)	0.34 (0.63)	0.253	0.33 (0.59)	0.32 (0.66)	0.816
Depressed mood (five items)	0.77 (1.35)	0.86 (1.37)	0.210	0.95 (1.56)	0.85 (1.34)	0.994	0.86 (1.32)	0.83 (1.35)	0.814
Questionnaire items of BCF									
Do you go out alone using transportation? (% no)	13.0	15.8	0.203	24.0	14.3	0.013	16.5	12.7	0.193
Do you shop for daily necessities by yourself? (% no)	4.9	8.6	0.021	17.3	7.1	0.001	8.6	5.9	0.198
Do you manage your bank account on your own? (% no)	10.5	11.7	0.553	18.3	10.5	0.024	12.9	8.6	0.094
Do you visit your friends alone? (% no)	17.2	16.7	0.842	22.1	15.7	0.107	16.1	15.4	0.832
Are you consulted by your family or friends? (% no)	12.7	9.8	0.132	15.4	8.8	0.038	8.2	9.3	0.666
Do you climb the stairs without holding on to handrails or walls? (% no)	22.8	32.9	<0.001	33.7	32.8	0.867	33.3	32.4	0.814
Do you stand up without assistance? (% no)	10.5	16.4	0.007	24.0	15.0	0.022	15.7	14.5	0.693
Can you walk for more than 15 min without rest? (% no)	6.6	13.0	0.001	15.4	12.6	0.443	12.9	12.4	0.841
Have you fallen within the past year? (% yes)	15.7	16.4	0.757	19.6	15.5	0.155	16.9	14.5	0.437
Are you anxious about falls? (% yes)	25.2	35.6	<0.001	42.3	34.4	0.120	33.7	34.8	0.772
Have you lost more than 2–3 kg in weight in the past 6 months? (% yes)	10.8	11.1	0.861	18.3	9.8	0.012	9.4	10.2	0.756
BMI $<18.5\,kg\,m^{-2}$ (% yes)	11.3	5.7	0.001	8.7	5.2	0.160	4.7	5.6	0.647
Do you have difficulty eating hard food? (% yes)	20.6	22.8	0.385	24.0	22.6	0.752	27.5	18.8	0.014
Do you choke when you swallow liquid? (% yes)	16.7	19.0	0.327	18.3	19.2	0.829	19.6	18.8	0.813
Do you have problems with a dry mouth? (% yes)	19.9	19.2	0.786	25.0	18.1	0.102	18.8	17.6	0.703
Do you go out more than once a week? (% no)	5.1	4.8	0.816	8.7	4.1	0.048	3.9	4.3	0.811
Do you go out less frequently than last year? (% yes)	15.6	19.3	0.121	21.6	18.9	0.526	19.4	18.4	0.760
Are you told that you repeatedly ask the same questions? (% yes)	12.3	15.7	0.120	20.2	14.9	0.168	14.1	15.4	0.659
Do you look up telephone numbers, dial and make phone calls without help? (% no)	4.4	4.5	0.922	10.6	3.5	0.001	2.7	4.0	0.407
Do you sometimes forget the date? (% yes)	13.0	16.4	0.128	19.2	15.9	0.397	16.5	15.4	0.734
Have you felt unfulfilled with daily life (in the last two weeks)? (% yes)	11.8	12.0	0.905	16.3	11.2	0.139	12.5	10.2	0.371
Have you not enjoyed your life as much as you used to (in the last 2 weeks)? (% no)	8.4	9.5	0.518	11.5	9.2	0.445	8.6	9.6	0.697
Do you feel more bothered about daily matters than you did before (in the last 2 weeks)? (% yes)	20.3	25.2	0.068	25.0	25.2	0.963	25.1	25.3	0.954
Have you felt that you are not useful (in the last 2 weeks)? (% no)	15.7	15.7	0.979	16.3	15.5	0.836	16.5	14.8	0.585
Have you felt tired for no reason (in the last 2 weeks)? (% yes)	21.4	23.9	0.344	26.0	23.5	0.586	23.5	23.5	0.984

Abbreviations: BP, blood pressure; BCF: basic checklist for frailty; CKD, chronic kidney disease. Results for continuous variables are expressed as mean ± s.d. and compared using Mann–Whitney U analysis. Discrete variables are reported as percentages and compared by χ^2-analysis.

status', besides absence of CKD or dyslipidemia, showed a statistically significant association with untreated hypertension in elderly hypertensive subjects (Table 2). In addition, logistic regression analysis using model-2 sets revealed that 'weight loss of more than 2–3 kg in the past 6 months' among these BCF items, besides absence of CKD or dyslipidemia, showed statistically significant association with untreated hypertension in elderly hypertensive subjects (Table 3).

Among treated hypertensive subjects, hypertensive subjects with uncontrolled BP (>140/90 mm Hg) showed a similar profile of

factors in baseline examinations and in BCF categories and items, except one BCF item, 'difficulty eating hard food', compared with hypertensive subjects with controlled BP in univariate analysis (Table 1). Logistic regression analyses revealed that 'impaired oral function' among the BCF categories in model-1 analysis (Table 2) and 'difficulty eating hard food' among the BCF items in model-2 analysis (Table 3) showed a statistically significant association with untreated hypertension in elderly community-dwelling subjects.

DISCUSSION

The present study newly disclosed an emerging profile of hypertension status and frailty in elderly community-dwelling subjects based on data from a Public Health Center survey and Regional Comprehensive Support Center in a town in Japan. The prevalence of hypertension (62.6%) in the present study in elderly community-dwelling Japanese subjects aged 65 years and older was comparable to the result (67%) in those aged 60 years and older in NHANES 1999–2004 in the US[6] and to that (≥50%) in those aged 75 years and older in Japan.[9] On the other hand, the rate of untreated hypertension in the present study, 15.2% of elderly hypertensive subjects aged 65 years and older, was rather low compared with the results in the US; 33% of those aged 60 years and older in NHANES 1999–2004[6] and 52% of those ≥18 years of age (mean age 58–60

Table 2 Results of BCF categories and clinical factors by multiple logistic analysis

	Wald	*OR*	*95%CI*	P
For hypertension among all subjects				
Age (years)	20.626	1.061	1.034–1.088	<0.001
Diabetes mellitus	10.048	1.862	1.268–2.736	0.002
Impaired nutritional status (two items)	8.300	0.645	0.479–0.869	0.003
Ischemic heart disease	3.332	2.288	0.940–5.568	0.068
Impaired walking status (five items)	3.269	1.107	0.991–1.236	0.070
Regular alcohol drinker	2.367	1.303	0.930–1.825	0.123
Dyslipidemia	2.296	1.272	0.931–1.737	0.129
Past history of stroke	1.499	1.473	0.792–2.740	0.220
Female sex	1.464	1.221	0.883–1.689	0.226
Current smoker	0.111	1.070	0.718–1.593	0.739
For treatment among hypertensive subjects				
Dyslipidemia	17.829	0.235	0.120–0.461	<0.001
CKD	9.754	0.438	0.261–0.735	0.002
Impaired IADL status (five items)	8.674	1.331	1.100–1.610	0.003
Impaired nutritional status (two items)	6.794	1.868	1.167–2.988	0.009
Current smoker	3.167	0.441	0.179–1.086	0.075
Diabetes mellitus	3.018	0.568	0.300–1.075	0.082
Female sex	2.239	1.534	0.875–2.689	0.134
Past history of stroke	1.499	1.677	0.733–3.836	0.220
Impaired walking status (five items)	0.080	0.974	0.813–1.166	0.776
Age (years)	0.042	1.004	0.963–1.047	0.836
For BP-control among treated hypertensive subjects				
Impaired oral function (three items)	3.957	1.236	1.003–1.523	0.047
Age (years)	3.790	0.968	0.933–1.001	0.053
Impaired IADL status (five items)	3.189	1.169	0.984–1.389	0.074
Dyslipidemia	3.036	0.712	0.486–1.043	0.081
Female sex	2.598	1.413	0.927–2.153	0.106
Past history of stroke	1.560	0.604	0.274–1.331	0.211

Abbreviations: BP, blood pressure; CI, confidence interval; CKD, chronic kidney disease; IADL, instrumental activity of daily living; OD, odds ratio. Adjusted by age, sex and variables selected according to their univariate analysis *P*-value ($P<0.20$).

Table 3 Results of BCF items and clinical factors by multiple logistic analysis

	Wald	*OR*	*95%CI*	P
For hypertension among all subjects				
Age (years)	23.338	1.067	1.039–1.096	<0.001
BMI $<18.5\,kg\,m^{-2}$	14.355	0.392	0.242–0.637	<0.001
Diabetes mellitus	9.821	1.863	1.262–2.749	0.002
Able to walk for more than 15 min (no)	4.345	1.732	1.033–2.904	0.037
Consulted by family or friends (no)	3.251	0.655	0.414–1.037	0.071
For treatment among hypertensive subjects				
Dyslipidemia	18.017	0.222	0.111–0.445	<0.001
CKD	9.699	0.434	0.257–0.734	0.002
Losing more than 2–3 kg in weight	7.546	2.518	1.302–4.868	0.006
Diabetes mellitus	3.802	0.526	0.275–1.003	0.051
Current smoking	2.889	0.450	0.179–1.130	0.089
Female sex	2.749	1.641	0.913–2.948	0.097
For BP-control among treated hypertensive subjects				
Difficulty eating hard food	6.283	1.690	1.121–2.548	0.012
Age (years)	3.683	0.968	0.938–1.001	0.055
Female sex	2.897	1.444	0.945–2.207	0.089
Able to manage bank account (no)	3.051	1.695	0.937–3.067	0.080

Abbreviations: BMI, body mass index; BP, blood pressure; CI, confidence interval; CKD, chronic kidney disease; OR, odds ratio. Adjusted by age, sex and variables selected according to their univariate analysis *P*-value ($P<0.20$). BCF items and clinical factors with multiple logistic analysis *P*-values ≥0.1 are not shown.

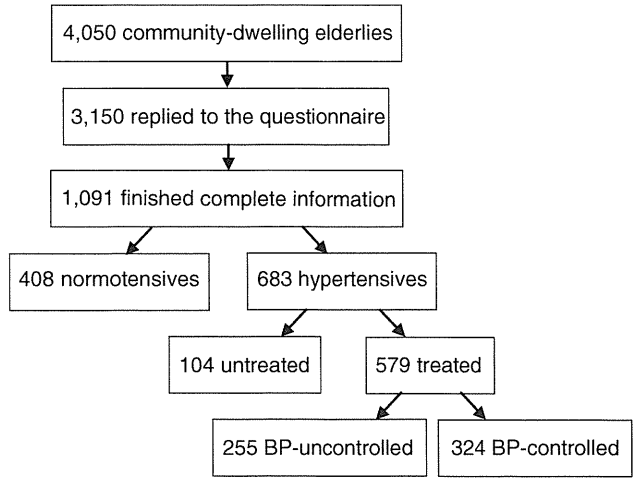

Figure 1 Study profile. Number of subjects per group shown for each status of hypertension.

years) in NHANES 1988–2008.[5] Moreover, the rate of BP-uncontrolled hypertension in the present study, 44% of treated hypertensive subjects aged 65 years and older, was also low compared with that in the US; 57% of those aged 60 years and older[6] and 53–73% of those ⩾18 years of age.[5] These differences may be reflected by the major fall of BP level recently achieved in community-dwelling subjects in Japan.[20]

Clinical factors independently associated with the prevalence of hypertension and with untreated hypertension in the present study were similar to those previously reported in community-dwelling subjects in the US, namely older age[6] and presence of diabetes mellitus[6] for prevalence of hypertension and absence of CKD[5,6] and absence of dyslipidemia[5] for untreated hypertension.

Logistic regression analysis in the present study also revealed that specified BCF categories and/or items were independently associated with hypertension status. First, two BCF categories, 'impaired walking status' and absence of 'impaired nutritional status', besides presence of diabetes mellitus, were independently associated with prevalence of hypertension in elderly subjects in logistic analysis using model-1 data sets. In addition, two BCF items, 'inability to walk for more than 15 min without a rest' and absence of 'BMI $<18.5\,kg\,m^{-2}$', were further shown to have an independent association with prevalence of hypertension in elderly subjects in logistic analysis using model-2 data sets. A possible explanation for the association of 'impaired walking status' or 'inability to walk for more than 15 min without rest' with prevalence of hypertension is that hypertension itself may cause physical frailty resulting in a decline in walking ability in the elderly, since elderly subjects with frailty syndrome with low physical activity had higher BP than the non-frailty group,[11] and since hypertension was independently associated with shorter distance on the 6-minute walk test in elderly subjects.[21] Another possible explanation is that daily practice of walking may prevent hypertension even in the elderly population, since subjects walking 1 hour or more per day had a lower prevalence of hypertension in a large population of frail and very old subjects living in the community.[22] On the other hand, the observation in the present study of the independent association of thinness (BMI $<18.5\,kg\,m^{-2}$) with lower prevalence of hypertension in the elderly is partly compatible with a previous report of an association of being underweight (BMI $<20\,kg\,m^{-2}$) with lower prevalence of hypertension in elderly subjects,[23] although it is well-known that BMI greater than the reference value ($25\,kg\,m^{-2}$) is independently associated with a greater likelihood of hypertension in the elderly.[6]

Second, two BCF categories, 'impaired IADL status' and 'impaired nutritional status', besides absence of CKD or dyslipidemia, were independently associated with untreated hypertension in hypertensive elderly subjects. The latter finding was further supported by the independent association of 'weight loss of more than 2–3 kg in the past 6 months' in model-2 logistic analysis using BCF items (Table 3). Although the precise mechanism of the association of 'impaired IADL status' with untreated hypertension in the elderly is unknown, IADL is a well-known indicator of the ability to live independently in the community. Okamura *et al.*[24] reported that elderly residents with systolic hypertension (⩾160 mm Hg) in two communities located in Akita and Kochi Prefectures showed a 3.41 times higher odds ratio for having low IADL scores than those with normal BP. Hayakawa *et al.*[25] reported a significant relationship between decrease in IADL score and cardiovascular risk factors including hypertension, dyslipidemia, diabetes mellitus and smoking, in a cohort in Japan. The present observation of an association between decline in IADL score and untreated hypertension is, at least in part, compatible with the reports of Okamura *et al.*[24] and Hayakawa *et al.*[25] Therefore, active treatment of hypertension in elderly community-dwelling subjects may be linked to prevention of future decline in IADL in Japanese elderly, allowing them to live a healthy and active life. On the other hand, the precise mechanism of the independent association of weight loss (of more than 2–3 kg in the past 6 months) with untreated hypertension in hypertensive elderly subjects is also unknown. One of the possible explanations for this is that weight loss as opposed to weight gain may often be overlooked as a problem linked to hypertension by healthcare providers, the public and elderly subjects themselves, as BMI $<25\,kg\,m^{-2}$ compared with BMI $\geqslant 25\,kg\,m^{-2}$ was reported to be independently associated with a greater likelihood of untreated hypertension in elderly subjects in the US.[6] Another possibility is that weight loss more often observed in elderly subjects with untreated hypertension might be caused by past antihypertensive drug treatment and result in cessation of drug treatment by elderly subjects themselves, as unintended weight loss in the elderly may be caused by polypharmacy through dysgeusia and anorexia due to many individual medications.[26]

Third, 'impaired oral function' in the BCF categories and 'difficulty eating hard food' in the BCF items were independently associated with BP-uncontrolled hypertension in treated hypertensive elderly subjects in respective logistic regression analysis models. One of the possible explanations for this is that oral dysfunction may directly cause trouble swallowing pills, resulting in underuse of antihypertensive medication in these subjects.[27] Another possibility is that periodontal disease may cause both 'difficulty eating hard food' and BP-uncontrolled hypertension. The severity of periodontal disease[28,29] and tooth loss due to the disease[30] were significantly related to hypertension independent of age, although inconsistent results were also reported in middle-aged men.[31] Moreover, periodontal disease is reported to contribute to poor BP control in subjects aged 70 years and older.[32]

In the present study, specified BCF categories and/or items were newly identified as factors independently associated with prevalence of hypertension, untreated hypertension and BP-uncontrolled hypertension in elderly community-dwelling subjects. These frailty categories and items may be useful for evaluating hypertension status in elderly community-dwelling subjects. However, in view of the single community model, care must be taken in interpreting these results, and further evaluation in multi-regional trials is needed. Frailty assessed by comprehensive geriatric assessments and a precise health examination should be included in future studies to elucidate the mechanisms of the individual associations of BCF categories/items and hypertension status. Stratified sampling of BCF scores according to the kinds of antihypertensive drugs used, including renin-angiotensin blockers, is also needed in future studies, because the renin-angiotensin system is thought to have a crucial role in aging and/or frailty.[33]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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1 Wolff JL, Starfield B, Anderson G. Prevalence, expenditures, and complications of multiple chronic conditions in the elderly. *Arch Intern Med* 2002; **162:** 2269–2276.
2 Insua JT, Sacks HS, Lau TS, Lau J, Reitman D, Pagano D, Chalmers TC. Drug treatment of hypertension in the elderly: a meta-analysis. *Ann Intern Med* 1994; **121:** 355–362.
3 Gueyffier F, Bulpitt C, Boissel JP, Schron E, Ekbom T, Fagard R, Casiglia E, Kerlikowske K, Coope J. Antihypertensive drugs in very old people: a subgroup meta-analysis of randomised controlled trials. INDANA Group. *Lancet* 1999; **353:** 793–796.
4 Beckett NS, Peters R, Fletcher AE, Staessen JA, Liu L, Dumitrascu D, Stoyanovsky V, Antikainen RL, Nikitin Y, Anderson C, Belhani A, Forette F, Rajkumar C, Thijs L, Banya W, Bulpitt CJ. Treatment of hypertension in patients 80 years of age or older. *N Engl J Med* 2008; **358:** 1887–1898.
5 Egan BM, Zhao Y, Axon RN, Brzezinski WA, Ferdinand KC. Uncontrolled and apparent treatment resistant hypertension in the United States, 1988–2008. *Circulation* 2011; **124:** 1046–1058.
6 Ostchega Y, Dillon CF, Hughes JP, Carroll M, Yoon S. Trends in hypertension prevalence, awareness, treatment, and control in older U.S. adults: data from the National Health and Nutrition Examination Survey 1988–2004. *J Am Geriatr Soc* 2007; **55:** 1056–1065.
7 Hajjar I, Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the United States, 1988–2000. *JAMA* 2003; **290:** 199–206.
8 Borzecki AM, Wong AT, Hickey EC, Ash AS, Berlowitz DR. Hypertension control: How well are we doing? *Arch Intern Med* 2003; **163:** 2705–2711.
9 Kuzuya M, Ando F, Iguchi A, Shimokata H. Age-specific change of prevalence of metabolic syndrome: longitudinal observation of large Japanese cohort. *Atherosclerosis* 2007; **191:** 305–312.
10 Wang L, van Belle G, Kukull WB, Larson EB. Predictors of functional change: a longitudinal study of nondemented people aged 65 and older. *J Am Geriatr Soc* 2002; **50:** 1525–1534.
11 Bastos-Barbosa RG, Ferriolli E, Coelho EB, Moriguti JC, Nobre F, da Costa Lima NK. Association of frailty syndrome in the elderly with higher blood pressure and other cardiovascular risk factors. *Am J Hypertens* 2012; **25:** 1156–1161.
12 Tsutsui T, Muramatsu N. Japan's universal long-term care system reform of 2005: Containing costs and realizing a vision. *J Am Geriatr Soc* 2007; **55:** 1458–1463.
13 Saito I, Kokubo Y, Yamagishi K, Iso H, Inoue M, Tsugane S. Diabetes and the risk of coronary heart disease in the general Japanese population: the Japan Public Health Center-based prospective (JPHC) study. *Atherosclerosis* 2011; **216:** 187–191.
14 Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999; **130:** 461–470.
15 Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A. On behalf of the collaborators developing the Japanese equation for estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53:** 982–992.
16 Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, Nanjo K, Sasaki A, Seino Y, Ito C, Shima K, Nonaka K, Kadowaki T. Committee of the Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract* 2002; **55:** 65–85.
17 Bando Y, Kanehara H, Aoki K, Katoh K, Toya D, Tanaka N. Characteristics of undiagnosed diabetes mellitus in a population undergoing health screening in Japan: target populations for efficient screening. *Diabetes Res Clin Pract* 2009; **83:** 341–346.
18 Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ito S, Iwao H, Kario K, Kawano Y, Kim-Mitsuyama S, Kimura G, Matsubara H, Matsuura H, Naruse M, Saito I, Shimada K, Shimamoto K, Suzuki H, Takishita S, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Ueshima H, Umemura S, Ishimitsu T, Rakugi H. Japanese Society of Hypertension Committee. The Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2009). *Hypertens Res* 2009; **32:** 3–107.
19 Concato J, Feinstein AR, Holford TR. The risk of determining risk with multivariable models. *Ann Intern Med* 1993; **118:** 201–210.
20 Ueshima H. Explanation for the Japanese paradox: prevention of increase in coronary heart disease and reduction in stroke. *J Atheroscler Thromb* 2007; **14:** 278–286.
21 Enright PL, McBurnie MA, Bittner V, Tracy RP, McNamara R, Arnold A, Newman AB. Cardiovascular Health Study. The 6-min walk test: a quick measure of functional status in elderly adults. *Chest* 2003; **123:** 387–398.
22 Landi F, Russo A, Cesari M, Pahor M, Liperoti R, Danese P, Bernabei R, Onder G. Walking one hour or more per day prevented mortality among older persons: results from ilSIRENTE study. *Prev Med* 2008; **47:** 422–426.
23 Barreto SM, Passos VM, Lima-Costa MF. Obesity and underweight among Brazilian elderly: the Bambuí Health and Aging Study. *Cad Saude Publica* 2003; **19:** 605–612.
24 Okamura T, Sato S, Kiyama A, Nakagawa Y, Naito Y, Iida M, Iso H, Shimamoto T, Komachi Y. Follow-up study on the relationship between the findings of cardiovascular screening, and prognosis for life and the capacity of activity in the elderly (65–74 years). *Kosei-No-Shihyou* 1977; **44:** 18–24. in Japanese.
25 Hayakawa T, Okamura T, Okayama A, Kanda H, Watanabe M, Kita Y, Miura K, Ueshima H. Relationship between 5-year decline in instrumental activity of daily living and accumulation of cardiovascular risk factors: NIPPON DATA90. *J Atheroscler Thromb* 2010; **17:** 64–72.
26 Huffman GB. Evaluating and treating unintentional weight loss in the elderly. *Am Fam Physician* 2002; **65:** 640–650.
27 Carnaby-Mann G, Crary M. Pill swallowing by adults with dysphagia. *Arch Otolaryngol Head Neck Surg* 2005; **131:** 970–975.
28 Holmlund A, Holm G, Lind L. Severity of periodontal disease and number of remaining teeth are related to the prevalence of myocardial infarction and hypertension in a study based on 4254 subjects. *J Periodontol* 2006; **77:** 1173–1178.
29 Morita T, Yamazaki Y, Mita A, Takada K, Seto M, Nishinoue N, Sasaki Y, Motohashi M, Maeno M. A cohort study on the association between periodontal disease and the development of metabolic syndrome. *J Periodontol* 2010; **81:** 512–519.
30 Al-Shammari KF, Al-Khabbaz AK, Al-Ansari JM, Neiva R, Wang HL. Risk indicators for tooth loss due to periodontal disease. *J Periodontol* 2005; **76:** 1910–1918.
31 Rivas-Tumanyan S, Spiegelman D, Curhan GC, Forman JP, Joshipura KJ. Periodontal disease and incidence of hypertension in the health professionals follow-up study. *Am J Hypertens* 2012; **25:** 770–776.
32 Rivas-Tumanyan S, Campos M, Zevallos JC, Joshipura KJ. Periodontal disease, hypertension and blood pressure among older adults in Puerto Rico. *J Periodontol* 2013; **84:** 203–211.
33 Abadir PM. The frail renin-angiotensin system. *Clin Geriatr Med* 2011; **27:** 53–65.

RESEARCH ARTICLE

Genome-wide response to antihypertensive medication using home blood pressure measurements: a pilot study nested within the HOMED-BP study

Background: Patients with mild-to-moderate essential hypertension in the HOMED-BP trial were randomly allocated to first-line treatment with a calcium channel blocker (CCB), angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB). **Methods:** We recruited 265 (93 for CCB, 71 for ACEI and 101 for ARB) patients who completed the genomic study. Home blood pressure was measured for 5 days off-treatment before randomization and for 5 days after 2–4 weeks of randomized drug treatment. Genotyping was performed by 500K DNA microarray chips. The blood pressure responses to the three drugs were analyzed separately as a quantitative trait. For replication of SNPs with $p < 10^{-4}$, we used the multicenter GEANE study, in which patients were randomized to valsartan or amlodipine. **Results:** SNPs in *PICALM*, *TANC2*, *NUMA1* and *APCDD1* were found to be associated with CCB responses and those in *ABCC9* and *YIPF1* were found to be associated with ARB response with replication. **Conclusion:** Our approach, the first based on high-fidelity phenotyping by home blood pressure measurement, might be a step in moving towards the personalized treatment of hypertension.

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KEYWORDS: antihypertensive drugs blood pressure response genome wide association study home blood pressure personalized treatment

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Only one-fourth of hypertensive patients take sufficient blood pressure control [1], this is because of inaccurate evaluation of blood pressure, inappropriate selection of antihypertensive drugs and poor patients' adherence to antihypertensive drug therapy [2]. Genetic factors also influence blood pressure elevation and response to antihypertensive medication [3]. To lead to personalized medicine based on genetic information, a genome-wide approach is desired in pharmacogenomics studies [4]. However, few studies have provided information on the response of crucial genes to various antihypertensive drugs by a comprehensive genome-wide association study (GWAS) approach [5–7].

Self-measurement of blood pressure at home using automated devices offers a greater number of readings that are free from observer bias. The home blood pressure measurement provides higher diagnostic accuracy compared with conventional office blood pressure measurement [8,9]. However, no previous pharmacogenomics study has assessed antihypertensive drug effect based on repeated home blood pressure measurements.

We carried out a GWAS in patients participating in the HOMED-BP study [10], a randomized controlled drug-intervention trial evaluating cardiovascular outcome based on home blood pressure measurement. In the present study, we aim to elucidate the genetic background underlying responsiveness to three major antihypertensive drugs, calcium channel blockers (CCBs), angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blocker (ARB).

Methods

Study protocol

The present HOMED-BP-GENE study is an ancillary project of the HOMED-BP study. Detailed protocol of the HOMED-BP Study has been described previously [10,11]. Briefly, patients with mild-to-moderate hypertension with a minimum age of 40 years were recruited from 457 general practices throughout Japan. Treatment naive patients as well as previously treated patients, whose antihypertensive drug treatment could be discontinued for at least 2 weeks, qualified for enrolment. Off treatment, they had to maintain a home blood pressure of 135–179 mmHg systolic or 85–119 mmHg diastolic. Eligible patients should have no contraindication for antihypertensive agents. Randomization was based on a computerized random number function with a minimization algorithm running on a central server at Tohoku University (Sendai, Miyagi, Japan), considering sex, age and the systolic and diastolic levels of the home blood pressure. In a 2 × 3 design, eligible patients

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were randomized to usual versus tight control of the home blood pressure and to the initiation of antihypertensive drug treatment with CCBs, ACEIs or ARBs. Usual control was a home blood pressure ranging from 125 to 134 mmHg systolic and from 80 to 84 mmHg diastolic. Tight control was home blood pressure values below 125 mmHg systolic and below 80 mmHg diastolic. The HOMED-BP protocol is registered with the UMIN Clinical Trial Registry, number C000000137 [101].

The HOMED-BP-GENE study aimed to elucidate the involvement of genetic influence focusing on SNPs. Physicians at 12 centers participated in the study, and 300 patients among 476 randomized patients from these centers were recruited for the genetic analysis. Of those, four patients were excluded because of protocol violation. Home blood pressure data during 10 to 28 days after the drug administration in 31 patients were not available or unreliable. Therefore, a total of 265 patients were analyzed in the present study (SUPPLEMENTARY FIGURE 1; www.futuremedicine.com/doi/suppl/10.2217/pgs.13.161). Owing to a bug in the electronic randomization, some patients were initially assigned to ARBs instead of ACEIs. In the pilot phase of the overall HOMED-BP study, this problem was identified and corrected, so that randomization became balanced. Because the patients included in the GWAS study were among those recruited early, there remains a shortfall in the number of patients randomized to ACEIs. All 265 patients analyzed patients took only a single antihypertensive agent during the period covered by the GWAS. Baseline characteristics of the 265 analyzed patients and 211 who did not take part in the GWAS project were similar ($p \geq 0.083$) except for age (61.5 vs 59.6 years; $p = 0.033$) and baseline systolic pressure (148.8 vs 152.2 mmHg; $p = 0.0039$). The study complies with the Declaration of Helsinki, and the study protocol was approved by the institutional review boards of Osaka University and Tohoku University, Japan. All study participants gave their written informed consent.

Evaluation of blood pressure changes

Patients received spoken and written instructions on blood pressure self-measurement and the utilized the validated [12] oscillometric OMRON HEM 747IC-N devices (Omron Healthcare Co., Ltd., Kyoto, Japan). They were asked to measure blood pressure and heart rate after 2 min of rest in a sitting position every morning throughout the whole study. They had to obtain these measurements within 1 h of waking up, before breakfast and before taking their antihypertensive medication. The device stores up to 350 readings in its memory. The home blood pressure values stored in the memory were uploaded via a local computer to the server at Tohoku University at each visit. These values were automatically calculated by the server and immediately displayed on the screen of the local computer in the practices along with an advice for treatment adjustment based on a computerized algorithm running on the central server.

Data on home morning blood pressure for 5 days before randomization and for 5 days after 10 to 28 days of randomized drug treatment were averaged separately. We used this time window for the following reasons: the home blood pressure used for determining eligibility and treatment adjustments at each visit in the HOMED-BP study was the average of the morning readings available over 5 days immediately preceding the visit [10,11]; the clinical investigators followed the study participants at intervals of approximately 2–4 weeks in general practice and 4–8 weeks at hospital outpatient clinics; and the time intervals needed to attain the maximum antihypertensive effects of losartan 50 mg, candesartan 8 mg, telmisartan 40 mg and olmesartan 20 mg on the morning blood pressure were 22.8, 11.0, 8.9 and 9.2 days, respectively [13]. In the genetic analysis, blood pressure response was calculated using the difference between these two periods. Differences in systolic and diastolic home blood pressure were analyzed individually.

Replication study – GEANE study

The GEANE study is a multicenter clinical trial registered in Japan (UMIN Clinical Trial Registry number C000000119 [101]), which utilized an open random crossover protocol to examine the antihypertensive effects of amlodipine (CCB, 5 mg/day), valsartan (ARB, 80 mg/day), and a thiazide-like diuretic indapamide (2 mg/day). After the written informed consent was approved by the ethical review committee of the National Cerebro- and Cardiovascular Research Center (Suita, Japan) and other collaborated institutes, order of drug prescription for each patient was decided by the automatic randomization system in the GEANE study administrative office. For the replication study of CCB- or ARB-responsive SNPs in the HOMED-BP-GENE study, we used 79 untreated patients in the GEANE study with essential hypertension of systolic office blood pressure ranging from 140 to 179 mmHg

and/or diastolic office blood pressure from 90 to 109 mmHg. Each assigned drug was prescribed for 3 months, starting at one half of the final dose and increasing to the regular dose. Home blood pressure was measured twice per day at home, and average values of 3 consecutive days before the randomization and at the end of each drug administration period, respectively, were used for the analysis. Blood samples at baseline were collected for checking basal medical status and genetic analyses.

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes and extracted from 200 ml of buffy coat using a QIAamp® DNA Blood Kit (Qiagen Inc., Hilden, Germany). All samples in the HOMED-BP-GENE study were genotyped by BIOMATRIX RESEARCH Co. Ltd. (Nagareyama, Chiba, Japan) with the Genome-Wide Human Mapping 500K SNPs Microarray (Affymetrix Genome Wide Human Array 5.0: Affymetrix, Inc. CA, USA) according to the manufacturer's instructions. Of the 443,816 SNPs on the array set, the number of SNPs suitable for the analysis was reduced to 298,046 (67.16%) for the following reasons (in order): were on the sex chromosome (n = 10,288), minor allele frequency <5% (n = 121,183), no call rate >10% across samples (n = 8028) and deviation from Hardy–Weinberg equilibrium p-value (<0.01; n = 6271).

Samples in the GEANE study were genotyped with the same Affymetrix Genome Wide Human Array 5.0 at the Research Institute of the National Cerebro- and Cardiovascular Research Center.

Statistical analyses

We used SAS software, version 9.3 (SAS Institute, NC, USA), for database management and basic statistical analysis. We also used JMP Genomics, version 6.0 (SAS Institute), for the phenotype–genotype analysis. For comparison of means and proportions, we applied the Z test for large samples and the χ^2 statistic, respectively. The genetic analysis was conducted for a trend-based and a genotype-based analyses at each SNP locus with covariate adjustment for age, sex, BMI, diabetes mellitus, the home blood pressure level before randomization, average duration after taking the treatment, and the defined daily doses (DDDs) of each drug [10,14,102]. DDD is the average maintenance dose per day for a drug administrated in its main indication [102].

We considered a p-value of $<10^{-5}$ as a statistically acceptable level based on the previous GWAS by the Wellcome Trust Case Control Consortium (WTCCC [15]), in which the same DNA microarray was used as in the present study [15]. We also reported association between blood pressure response and SNPs with p-values from 10^{-4} and 10^{-5}. In the GEANE replication study, we performed similar trend-based and genotype-based analyses for SNP locus with covariate adjustment for age, sex, BMI and home blood pressure level before randomization. We did not conduct the replication study for SNPs associated with an ACEI response because the GEANE study did not include patients with an ACEI prescription.

Results

Patient characteristics in all analyzed subjects are shown in Table 1. Although the number of patients in the ACEI group (n = 71), was smaller than those in CCB and ARB groups (n = 93 and 101, respectively), baseline characteristics before treatment did not differ among the three drug groups ($p \geq 0.075$) with the exception of age ($p = 0.0074$).

Home blood pressure responses in the ACEI group were significant but small (systolic: -3.7 ± 9.9 mmHg, p = 0.0022; diastolic: -2.4 ± 6.0 mmHg, p = 0.0014) compared with those in the CCB group (systolic: -9.4 ± 10.4 mmHg, p < 0.0001; diastolic: -4.1 ± 6.2 mmHg, p < 0.0001) and in the ARB group (systolic: -7.8 ± 10.3 mmHg, p < 0.0001; diastolic: -3.6 ± 5.5 mmHg, p < 0.0001). Figure 1 & Supplementary Figure 2 show Manhattan plots from the GWAS indicating associations between 298,046 SNPs and home blood pressure response to CCB, ACEI and ARB using trend tests (Figure 1) and genotype tests (Supplementary Figure 2), respectively. Of those, 16 SNPs and 284 SNPs were associated with the antihypertensive effect for home blood pressure by CCB, ACEI or ARB with p-value less than 10^{-5} and 10^{-4}, respectively (Supplementary Tables 1 & 2).

Clinical baseline characteristics of the 79 GEANE replication study participants are shown in Supplementary Table 3. No significant treatment effect as well as carry-over effect and period effect were detected in any of the three drug arms for home blood pressure (p = 0.14, 0.98 and 0.53 for systolic pressure; p = 0.49, 0.92 and 0.85 for diastolic pressure, respectively). Including those with marginal significance (p < 0.10), replicated SNPs associated with drug response are shown in Table 2. Six associated gene polymorphisms were

Table 1. Characteristics of HOMED-BP-GENE participants.

Characteristic	CCBs	ACEIs	ARBs
Number	93	71	101
Mean characteristic, mean (SD)			
Age (years)	59.6 (9.7)	60.7 (8.9)	63.7 (9.5)
BMI (kg/m^2)	24.8 (3.1)	25.0 (3.1)	24.5 (3.0)
Home measurements before treatment, mean (SD)			
– Systolic pressure (mmHg)	148.9 (13.9)	149.3 (11.4)	148.3 (12.2)
– Diastolic pressure (mmHg)	89.7 (9.9)	89.7 (9.3)	86.8 (10.4)
– Heart rate (bpm)	67.1 (8.6)	67.1 (8.5)	67.4 (9.8)
Home measurements after drugs, mean (SD)			
– Systolic pressure (mmHg)	139.5 (12.7)	145.5 (13.7)	140.5 (14.8)
– Diastolic pressure (mmHg)	85.6 (9.3)	87.3 (10.3)	83.1 (10.2)
– Heart rate (bpm)	68.5 (8.4)	66.1 (9.0)	68.2 (11.0)
Biochemical measurements, mean (SD)			
– Plasma glucose (mmol/l)	5.9 (1.7)	5.8 (0.9)	5.9 (1.3)
– Serum total cholesterol (mmol/l)	5.3 (0.8)	5.3 (0.9)	5.4 (0.8)
Number with characteristic, proportion (%)			
Women	53 (57.0)	37 (52.1)	53 (52.5)
Current smoking	19 (20.4)	13 (18.3)	19 (19.0)
Habitual drinking	48 (51.6)	37 (52.1)	58 (58.0)
Diabetes mellitus	9 (9.7)	6 (8.5)	18 (17.8)
Hypercholesterolemia	33 (35.5)	18 (25.4)	32 (31.7)
Previous cardiovascular disease	5 (5.4)	4 (5.6)	7 (6.9)

Home measurements are the average of the morning readings over 5 days immediately preceding the clinic visit. Diabetes mellitus is defined as a fasting plasma glucose level of 7.0 mmol/l (126 mg/dl) or more, or a HbA_{1c} level of 6.5% or more, or treatment with oral antidiabetic drugs or insulin. Hypercholesterolemia is defined as a total cholesterol level of 5.69 mmol/l (220 mg/dl) or more, a history of hypercholesterolemia or taking lipid-lowering drugs. Baseline characteristics before treatment did not differ among three drug groups ($p \geq 0.075$) with the exception of age ($p = 0.0074$).
ACEI: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin II receptor blocker; bpm: Beats per minute; CCB: Calcium channel blocker; SD: Standard deviation.

further analyzed for blood pressure reduction by corresponding antihypertensive drug classes (Table 3). As a representative, two tightly linked SNPs, rs1283807 and rs704209 (Supplementary Figure 3), are located in the intron of *ABCC9*, and systolic home blood pressure response to ARB was significantly different among the three genotypes in rs1283807, G/G (-12.7 ± 9.1 mmHg), A/G (-8.2 ± 9.3 mmHg) and A/A (+2.1 ± 9.7 mmHg). Other associated genes for systolic blood pressure response were *PICALM* by tightly linked SNPs (rs588076 and rs597446; Supplementary Figure 4) and *TANC2*. Those for diastolic blood pressure response were *YIPF1* by completely linked SNPs (rs6680026 and rs6588492; Supplementary Figure 5), *NUMA1* and *APCDD1*.

We carried out a sensitivity analysis including all morning blood pressure measurements available from 10 to 28 days after the randomization. These results were confirmatory.

Discussion

This study is the first GWAS with replication to clarify the SNPs susceptible to the antihypertensive effects of CCBs, ACEIs and ARBs using repeated home blood pressure measurements. In consequence, SNPs in *PICALM, TANC2, NUMA1* and *APCDD1* are associated with CCB responses and those in *ABCC9* and *YIPF1* are associated with ARB responses.

Previously, there was no genome-wide pharmacogenomic study for the effect of antihypertensive drugs using home blood pressure measurements. Repeated home blood pressure measurements are more accurate than conventional blood pressure measurements [8,9]. The reproducibility of home blood pressure, defined as the difference between the initial 5-day average (4–8 days) and the last 5-day average (17–21 days), was -1.9 ± 7.0 (mean ± standard deviation) mmHg for systolic

and -1.4 ± 4.8 mmHg for diastolic, respectively, among 172 patients from a general population in Ohasama, Japan [16]. In the clinical setting, the corresponding difference among 42 patients with placebo administration was 1.1 ± 6.2 mmHg for systolic and 0.2 ± 5.7 mmHg for diastolic, respectively [16]. Thus, the home blood pressure measurement is highly reproducible and shows that there is minimal, possible no, placebo effect. Compared with conventional blood pressure, we require a smaller number of subjects to evaluate the antihypertensive effect of a drug treatment based on home blood pressure [16].

Turner and colleagues investigated the blood pressure response to candesartan by validating opposite direction associations with the response to hydrochlorothiazide [7]. Based on a case–control sampling design, each of the 300 white patients (in Rochester, MN, USA) and 300 black patients (in Atlanta, GA, USA) who were treated with candesartan were divided into three groups according to their diastolic blood pressure responses, and mid-tertiles were eliminated (102 white patients and 107 black patients, respectively). Turner and colleagues carried out the primary genome-wide association analyses of categorically defined good versus poor blood pressure response to candesartan. By SNP p-values $<10^{-4}$, they identified 285 SNPs in white patients and 272 SNPs in black patients that were associated with the response to candesartan. They identified six SNPs that had the most significant opposite direction associations with blood pressure response to

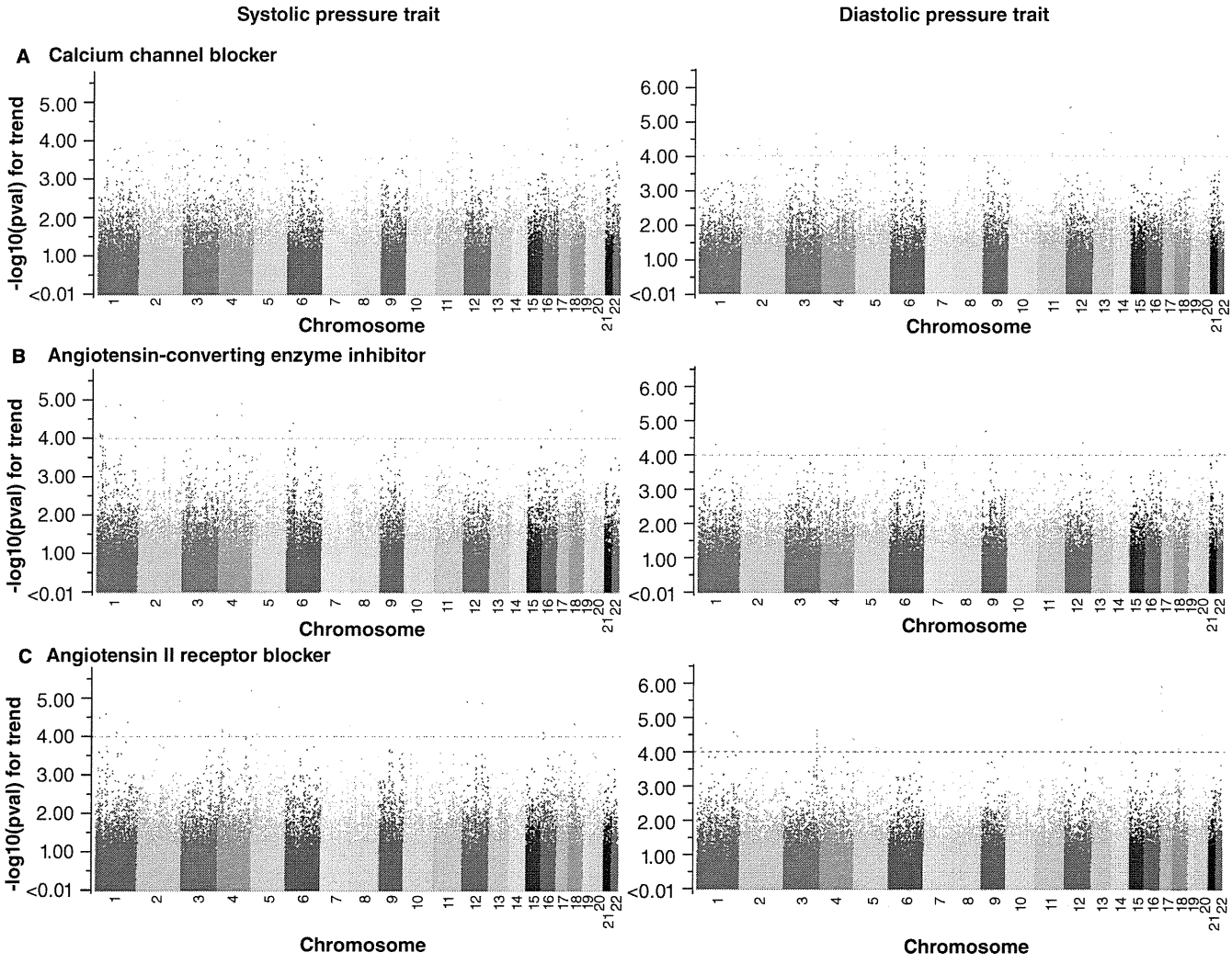

Figure 1. Manhattan plots showing genome-wide association results of SNPs with home blood pressure response to a calcium channel blocker, angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker. (A) Calcium channel blocker, **(B)** angiotensin-converting enzyme inhibitor and **(C)** angiotensin II receptor blocker. The p-values for the 298,046 SNPs on autosomes obtained using trend tests in systolic (left panels) and diastolic (right panels) home blood pressure. The horizontal dashed line indicates the threshold of $p < 10^{-4}$.

Table 2. SNPs associated with the response to calcium channel blocker and angiotensin II receptor blocker with replication $p < 0.10$.

Chromosome	dbSNP rs ID	Position	Associated gene (intron)	Allele		MAF	p-values			
				Responsive	*Not responsive*		*Genotype*	*Trend*	*Replication*	
									Genotype	*Trend*
Calcium channel blocker: systolic pressure trait										
1q25	rs4652519	180549164	NA	G	A	0.102	6.73×10^{-5}	7.64×10^{-1}	0.089	0.089
2q35	rs1949872	217443802	NA	G	A	0.052	9.80×10^{-5}	9.80×10^{-5}	0.074	0.074
3p24	rs12494691	16700334	NA	G	A	0.194	6.61×10^{-5}	4.00×10^{-1}	0.10	**0.052**
11q14	rs588076	85691662	*PICALM*	C	G	0.290	7.41×10^{-5}	5.64×10^{-4}	0.047	0.019
11q14	rs597446	85775059	*PICALM*	G	A	0.289	8.22×10^{-5}	5.79×10^{-4}	0.099	0.037
11q14	rs541458	85788351	NA	C	T	0.473	3.87×10^{-4}	9.10×10^{-5}	0.034	0.051
16p13	rs7196683	10924058	NA	G	A	0.140	1.22×10^{-5}	2.11×10^{-1}	0.035	0.035
17q23	rs2429427	61393816	*TANC2*	G	A	0.135	2.75×10^{-5}	2.75×10^{-5}	0.055	0.061
17q23	rs1029765	61459284	*TANC2*	A	G	0.135	2.75×10^{-5}	2.75×10^{-5}	0.072	0.081
Calcium channel blocker: diastolic pressure trait										
1p22	rs6576888	87746317	NA	G	T	0.295	4.30×10^{-5}	5.68×10^{-1}	0.13	0.047
1p22	rs6675584	87752776	NA	A	C	0.295	7.01×10^{-6}	5.63×10^{-1}	0.18	0.078
1p22	rs6688319	87752886	NA	C	A	0.297	3.52×10^{-5}	5.23×10^{-1}	0.18	0.078
4q32	rs7683367	162135488	NA	T	C	0.113	1.47×10^{-4}	4.15×10^{-5}	0.069	0.069
5q34	rs10516010	165654695	NA	T	A	0.172	2.51×10^{-5}	3.89×10^{-6}	0.12	**0.051**
6q26	rs6928944	164351521	NA	G	A	0.202	2.46×10^{-4}	6.02×10^{-5}	0.0010	0.26
11q13	rs10898815	71739423	*NUMA1*	C	T	0.183	1.72×10^{-4}	8.64×10^{-5}	0.0089	**0.033**
18p11	rs564991	10465054	*APCDD1*	C	A	0.442	9.14×10^{-5}	2.48×10^{-1}	0.017	**0.0059**
Angiotensin II receptor blocker: systolic pressure trait										
12p12	rs704209	22042560	*ABCC9*	A	C	0.455	3.64×10^{-5}	1.27×10^{-5}	0.070	0.15
12p12	rs1283807	22042740	*ABCC9*	G	A	0.455	3.64×10^{-5}	1.27×10^{-5}	0.061	0.15
13q34	rs9560161	112338725	NA	G	A	0.176	4.82×10^{-6}	3.31×10^{-2}	0.045	**0.015**
Angiotensin II receptor blocker: diastolic pressure trait										
1p32	rs6588492	54328025	*YIPF1*	T	C	0.120	4.23×10^{-5}	1.51×10^{-5}	0.015	0.0037
1p32	rs6680026	54345792	*YIPF1*	T	C	0.120	4.23×10^{-5}	1.51×10^{-5}	0.015	0.0037
4p15	rs4575988	19984926	NA	A	G	0.202	3.90×10^{-4}	7.50×10^{-5}	0.0095	0.15
5p15	rs314022	18319588	NA	G	C	0.422	9.92×10^{-5}	8.96×10^{-2}	0.049	0.37
5q31	rs7730672	135969163	NA	T	A	0.263	3.27×10^{-4}	8.36×10^{-5}	0.18	0.085

Bold values are opposite direction of allele tested for trend $p < 0.1$.
MAF: Minor allele frequency; NA: Not applicable.

Table 3. Blood pressure reduction in associated genes by antihypertensive drug classes.

Associated gene	Major homozygous	Heterozygous	Minor homozygous	Unadjusted p-values		Adjusted p-values	
				Genotype	*Trend*	*Genotype*	*Trend*
Calcium channel blocker:* PICALM *(rs588076)							
Genotype, n	C/C: 46	C/G: 41	G/G: 6				
δ SBP (mmHg)	-11.4 (10.3)	-9.6 (8.3)	7.1 (10.8)	0.0001	0.0015	<0.0001	0.0006
δ DBP (mmHg)	-5.7 (6.0)	-3.1 (5.8)	1.9 (6.2)	0.0077	0.0024	0.0012	0.0013
Calcium channel blocker:* TANC2 *(rs2429427)							
Genotype, n	G/G: 69	G/A: 24	A/A: 0				
δ SBP (mmHg)	-11.0 (9.2)	-4.9 (12.5)	–	0.013	0.013	<0.0001	<0.0001
δ DBP (mmHg)	-4.8 (6.2)	-2.1 (5.9)	–	0.067	0.067	0.0035	0.0035
Calcium channel blocker:* NUMA1 *(rs10898815)							
Genotype, n	C/C: 63	C/T: 28	T/T: 2				
δ SBP (mmHg)	-11.2 (9.4)	-5.4 (12.1)	-10.7 (0.4)	0.051	0.040	0.12	0.12
δ DBP (mmHg)	-5.8 (5.7)	-0.4 (6.0)	-2.2 (2.5)	0.0004	0.0002	0.0002	<0.0001
Calcium channel blocker:* APCDD1 *(rs564991)							
Genotype, n	C/C: 30	C/A: 46	A/A: 17				
δ SBP (mmHg)	-8.0 (10.0)	-11.7 (11.2)	-5.8 (7.7)	0.095	0.79	0.016	0.39
δ DBP (mmHg)	-1.5 (5.3)	-6.7 (5.9)	-1.5 (5.9)	0.0002	0.44	<0.0001	0.25
Angiotensin II receptor blocker:* ABCC9 *(rs1283807)							
Genotype, n	G/G: 28	G/A: 57	A/A: 16				
δ SBP (mmHg)	-12.7 (9.1)	-8.2 (9.3)	2.1 (9.7)	<0.0001	<0.0001	<0.0001	<0.0001
δ DBP (mmHg)	-6.2 (6.6)	-3.5 (4.2)	0.4 (5.1)	0.0004	<0.0001	0.0012	0.0003
Angiotensin II receptor blocker:* YIPF1 *(rs6588492)							
Genotype, n	T/T: 84	T/C: 16	C/C: 1				
δ SBP (mmHg)	-7.4 (9.9)	-9.0 (12.3)	-23.2 (–)	0.28	0.24	0.27	0.13
δ DBP (mmHg)	-2.9 (5.3)	-6.7 (4.6)	-18.4 (–)	0.0008	0.0004	<0.0001	<0.0001

Data are displayed as mean (standard deviation). The covariables in adjusted model were sex, age, BMI, baseline blood pressure level, diabetes mellitus, duration of taking drugs and the defined daily doses of each drug. DBP: Diastolic home blood pressure; SBP: Systolic home blood pressure.

hydrochlorothiazide (i.e., one-sided $p < 0.05$), but only from 273 of the 285 SNPs in white patients because the χ^2 test statistic in black patients showed nonsignificant results for the associations. Those in the chromosome 11q21 region were the most significantly associated with response to candesartan in white patients, had the strongest opposite direction associations with response to hydrochlorothiazide and had the same direction associations with response to candesartan in the 193 black patients. Their approach, however, does not wholly substantiate the conclusions. First, they analyzed only diastolic blood pressure response, not systolic pressure. Second, no rationale was found why they eliminate the mid-tertiles of blood pressure response in a categorical analysis, this could have resulted in the loss of information. Finally, there is little evidence as to whether or not the opposite direction blood pressure response to ARB, candesartan and diuretic hydrochlorothiazide would be observed in clinical practice.

PICALM, also known as CALM, is involved in clathrin-mediated endocytosis and is ubiquitously expressed in all tissues [17,18]. SNPs in the *PICALM* gene were reported to be significantly associated with Alzheimer's disease. [18,19]. The role of *PICALM* in the pathogenesis of Alzheimer's disease could be explained through the following: a possible role of clathrin-mediated endocytosis in internalization and recycling of full length amyloid precursor protein [19]; PICALM's role in synaptic vesicle formation; reduction of synaptic density and memory loss in Alzheimer's disease [20]; and dendritic dystrophy and disrupted endocytosis and secretion of neurotransmitters owing to PICALM reduction as seen in cell culture [21]. Based on the established association of late-onset Alzheimer's disease with the ε4 variant of APOE [22], Sweet and colleagues reported that genetic variation in *PICALM* was also associated with lower age at midpoint of cognitive decline in models that included apolipoprotein E [23]. From the double-blind, placebo-controlled Syst-Eur trial, blood pressure lowering therapy initiated with nitrendipine, a long-acting CCB, protects against dementia in older hypertensive patients [24,25]. The protective effect of CCB for cognitive function might be mediated by gene variations, including *PICALM*.

Although marginally replicated, two SNPs in *ABCC9* (rs704209 and rs1283807) showed strong association with the response to ARB. *ABCC9* encoded ATP-sensitive potassium channels are characterized by inhibition of channel opening when the ATP concentration at the cytoplasmic cell surface is increased. *ABCC9* is highly expressed in skeletal muscle and the heart and regulates ATP-sensitive potassium channels [26]. Inagaki and colleagues noted that sulfonylureas, substances widely used as oral hypoglycemic agents in the treatment of noninsulin-dependent diabetes mellitus, inhibits the activity of potassium channels [27]. They cloned *ABCC9* known as an isoform of a sulfonylurea receptor, designated sulfonylurea receptor-2, from a rat brain cDNA library [28]. Genetic mutations of *ABCC9* are reported to be one of the causes in dilated cardiomyopathy [29] and familial atrial fibrillation [30]. From the meta-analysis based on 11 randomized controlled trials, Healey and colleagues reported that ARB as well as ACEI has a protective effect beyond blood pressure reduction for atrial fibrillation [31]. For ACEI, this effect was most clearly observed in patients with left ventricular dysfunction [32] and clinical heart failure [33]. Although detailed mechanisms of *ABCC9* connected to the antihypertensive effects of ARBs are unclear and no previous study reported on the association between *ABCC9* and the renin–angiotensin system, genetic alteration by SNPs in *ABCC9* may influence on the pharmacological action of ARBs.

Recent findings by Shimomura and colleagues demonstrated that *APCDD1* could regulate a diversity of biological processes controlled by Wnt signaling [34]. Wnt signaling is involved in heart development and is reported to be activated in cardiac hypertrophy and cardiomyopathy [35,36]. *In vitro*, Wnt-5a effect on field excitatory postsynaptic potentials in hippocampal slices was blocked by nifedipine [37]. *APCDD1* polymorphisms might affect the electrophysiological activity of the heart, although it remains conjectural.

To the best of our knowledge, the current study is the first report to demonstrate the clinical importance of two SNPs in *YIPF1* (rs6588492 and rs6680026) that are associated with ARB response. These SNPs were strongly associated in the HOMED-BP population ($p \leq 4.23 \times 10^{-5}$) and also significantly replicated for both genotype and trend associations in the GEANE population ($p \leq 0.015$). There is only one report regarding the *TANC2* gene. The coding protein TANC2 is differentially expressed in postmortem brains from patients with atypical cases of frontotemporal lobar degeneration, but the metabolism of TANC2 is unknown [38]. The *NUMA1* gene maps to chromosome 11q13 [39],