第７回　GEANE研究打ち合わせ会

日　時：　平成１９年8月２５日　１４:００-１６:００
場　所：　千里阪急ホテル　西館３階　梅桃の間）

出席予定者（敬称は略させていただきました）：

大阪大学老年・腎臓内科学	杉本　研
国立病院機構九州医療センター高血圧内科	土橋卓也
国立病院機構福岡東医療センター	上野道雄、片渕律子
日本大学医学部	相馬正義
日本赤十字社長崎原爆病院	品川達夫
金沢医大高齢医学	森本茂人、中橋　毅
愛媛大学老年医学	三木哲郎、伊賀瀬道也
済生会呉病院	松浦秀夫
順天堂大学	佐瀬一洋
川崎医科大学	冨田奈留也
東宝塚さとう病院	滝内　伸

国立循環器病センター　河野雄平、花田裕典、神出　計、嘉田晃子、安田久代、西田秀範、松本幸子、山本勝広、上紙委千代、早瀬えるむ

１．開会挨拶
　　国立循環器病センター　　高血圧腎臓内科　河野雄平
２．各施設での近況報告
　　司会：国立循環器病センター　　高血圧腎臓内科　神出　計
３．臨床研究を進める上での問題点等のディスカッション
　a. プロトコールの確認、問題点
　b. 患者登録・割付
　c. 検体採取、処理、移送について
　d. 研究を進める上での今後の課題
４．臨床情報、データベース
５．遺伝子解析の現況
　　国立循環器病センター　研究所　花田裕典
６．研究費、事務連絡
７．閉会挨拶

第８回 GEANE 研究打ち合わせ会

日時:平成 20 年 2 月 16 日　午前 11 時～午後 3 時

場所:国立循環器病センター　新館講堂

出席予定者

(敬称は略させていただいております。誤りや変更がございましたらお知らせ下さい)

大阪大学老年・腎臓内科	勝谷友宏・杉本　研
国立病院機構九州医療センター	土橋卓也
国立病院機構福岡東医療センター	上野道雄・片渕律子
九州大学臨床薬理学	笹栗俊之・三輪宜一
金沢医科大学高齢医学	森本茂人
日本赤十字社長崎原爆病院	品川達夫
愛媛大学加齢制御内科学	伊賀瀬道也
獨協医科大学循環器内科	石光俊彦
川崎医科大学腎臓内科	冨田奈留也・駒井則夫
済生会呉病院	松浦秀夫
京都工場保健会	武田和夫
順天堂大学	佐瀬一洋
東京大学医学部附属病院	下澤達雄
東宝塚さとう病院	滝内　伸
統計数理研究所	藤田利治・江口真透
国立循環器病センター	友池仁暢、河野雄平、宮田敏行、花田裕典、神出　計、嘉田晃子、安田久代、松本幸子、山本勝広、上紙委千代、早瀬えるむ

(敬称は略させていただいております。)

司　会:　国立循環器病センター　高血圧腎臓内科　神出　計

1. 開会挨拶　国立循環器病センター　高血圧腎臓内科　河野雄平
2. 各施設での近況報告・調査票の回収状況など　神出　計
3. 臨床研究を進める上での問題点等のディスカッション
   a. プロトコールの確認、問題点
   b. 患者登録・割付・フォローアップ
   c. 研究を進める上での今後の課題
4. 臨床情報、データベース
5. 遺伝子解析・データベース構築の現況(ゲノム網羅的解析)
   国立循環器病センター研究所　花田裕典
6. 遺伝子解析の現況(候補遺伝子解析)
   国立循環器病センター研究所・高血圧腎臓内科　松本幸子
7. 統計解析ストラテジー(仮題)　統計数理研究所　江口真透
8. 研究費、事務連絡
9. 閉会挨拶　河野雄平

平成19年8月25日
国立循環器病センター

第7回研究打ち合わせ会

厚生労働科学研究

降圧薬感受性遺伝子同定のための多施設前向き臨床試験

GEANE

Gene Evaluation for ANtihypertensive Effect of drugs

最近の主な動き

- 平成19年3月2日　19年度厚生労働科研継続決定通知あり（創薬基盤推進研究事業:ヒトゲノムテーラーメード研究）
- 平成19年3月3日　第6回GEANE会議
- 平成19年3月30日　交付額通知あり　44,639,000円（うち間接経費10,301,000円）
- 平成19年3月31日　症例登録締め切り
- 平成19年4月6日　平成18年度報告書提出
- 平成19年4月18日　平成19年度交付申請書提出
- 平成19年6月21日　平成19年度交付決定通知あり

主な研究者の追加・変更

新たに参加していただく研究者の方
- 統計数理研究所　藤田利治教授　（データ解析）
- 統計数理研究所　江口真透教授　（データ解析）
- 国立循環器病センター研究所　松本幸子研究員（遺伝子解析）

共同研究機関における変更
- 大阪大学　荻原教授の御退官により研究責任者が勝谷友宏先生に変更。
- 長崎神経医療センター　品川先生が日赤長崎原爆病院に異動。後任は吉田和郎先生。
- 札幌医大　東浦先生の異動に伴い、研究担当者が宮崎先生に変更。

登録患者数（3/31/07登録締め切り）

	登録数	男	女	中止・脱落数
国循センター	63	41	22	3
金沢医科大学	14	7	7	0
京都桂病院	14	9	5	(1)
福島医科大学	8	2	6	1
九州医療センター	7	5	2	0
白大	6	2	4	1
済生会泉病院	5	2	3	0
愛媛大学第二内科	5	0	5	(1)
長崎神経医療	5	2	3	0
一本松病院	3	0	3	0
愛媛大学老年	3	3	0	0
大阪大学	3	2	1	0
香川大学	3	2	1	2
勝谷病院	3	2	1	0
京都工場保健会診療所	3	2	1	0
札幌医科大学	3	3	0	1
福岡東医療センター	3	1	2	2
宮崎大学	1	0	1	0
川崎医科大学	1	0	1	0
静岡医療センター	1	1	0	0
計	154	88	88	10(12)

登録患者背景

全登録患者数	154例
男:女	86:68
平均年齢（全体）	58．3±12．3歳
平均年齢（男性）	56．2 ±13．1歳
平均年齢（女性）	60．9 ±10．6歳

（中止・脱落例を含む）

各施設の近況報告

プロトコール、患者割付

割付の内訳

投薬順序	割付数	中止・脱落	
ナトリックス→ディオバン→ノルバスク	27	2	25
ナトリックス→ノルバスク→ディオバン	27	3	24
ディオバン→ナトリックス→ノルバスク	28	2	26
ディオバン→ノルバスク→ナトリックス	26	1	25
ノルバスク→ナトリックス→ディオバン	23	2	21
ノルバスク→ディオバン→ナトリックス	23	0	23
計	154	10	144

中止・脱落の理由

- 同意の撤回　1件（投薬・DNA採血前）
- 観察期間に重症高血圧となり降圧薬による治療が必要となったため。 1件　(DNA採血前)
- 登録直後より来院なし。 3件（2件は採血済）
- 登録後第2薬投与から来院なし。 2件（1件は転居による。いずれも採血済）
- 経過観察中(第1薬投与中)に悪性腫瘍あり。　1件 (DNA採血済)
- 第1薬インダパミド1mgで頻尿・動悸・目の前が黄色くなるなどの症状出現。第2薬への変更も拒否。 1件 (DNA採血済)
- 観察期の家庭血圧が正常。投薬の必要性が少ないと判断された。 1件　(DNA採血前)

遺伝子検体の集積状況

遺伝子検体受領数

	登録数	男	女	中止・脱落数	検体受領数
国循センター	63	41	22	3	62
金沢医科大学	14	7	7	0	13
東京逓信病院	14	9	5	(1)	13
獨協医科大学	8	2	6	1	7
九州医療センター	7	5	2	0	7
日大	6	2	4	1	5
済生会吹田病院	5	2	3	0	5
愛媛大学第二内科	5	0	5	(1)	4
長崎神経医療	5	2	3	0	4
一本松病院	3	0	3	0	3
愛媛大学老年	3	3	0	0	3
大阪大学	3	2	1	0	3
香川大学	3	2	1	2	3
循環器病	3	2	1	0	3
京都工場保健会診療所	3	2	1	0	3
札幌医科大学	3	3	0	1	2
福岡東医療センター	3	1	2	2	3
宮崎大学	1	0	1	0	1
川崎医科大学	1	0	1	0	1
静岡医療センター	1	1	0	0	1
計	154	88	66	10(12)	148

DNAの共同使用

国循ではGEANE研究のDNA共同使用を歓迎いたします。御興味のある遺伝子多型などとGEANEの3種降圧薬の効果を確認するような研究を御計画でしたらDNAを分与いたしますのでGEANE研究サブ解析として御検討ください。研究期間内により多くの研究成果を出すことが研究の高評価に繋がります。

調査票の集積状況

調査票回収状況

- 国循センター　　49件
- 他施設　　21件

調査票受領数

	登録数	男	女	中止・脱落数	調査票受領数
国循センター	63	41	22	3	49
金沢医科大学	14	7	7	0	0
東北厚生年金病院	14	8	5	(1)	0
順天堂医科大学	8	2	6	1	6
九州医療センター	7	5	2	0	4
日大	6	2	4	1	5
済生会長岡病院	5	2	3	0	3
愛媛大学第二内科	5	0	5	(1)	0
長崎神経医療	5	2	3	0	0
一本松病院	3	0	3	0	2
愛媛大学老年	3	3	0	0	0
大阪大学	3	2	1	0	0
香川大学	3	2	1	2	0
勝谷医院	3	2	1	0	0
京都工場保健会診療所	3	2	1	0	0
札幌医科大学	3	3	0	1	0
福岡東医療センター	3	1	2	2	0
宮崎大学	1	0	1	0	1
川崎医科大学	1	0	1	0	0
静岡医療センター	1	1	0	0	0
計	154	88	68	10(12)	70

データベース

1. 調査票の情報をFILE MAKER PROに入力
2. EXCELに変換
3. NCVC分は匿名化
   他施設分は匿名化済み

遺伝子解析の現状

国立循環器病センター研究所

花田裕典

平成19年度の研究計画

GeneChip のデータ解析について
- ・作製(委託)した遺伝子型データベースソフトウェアに現在データを登録中
- ・文献情報を元にした候補遺伝子について関連性解析　高血圧学会で発表予定
- ・コピーナンバー解析による欠損領域の検出(解析ソフトの関連性解析)
- ・生物・医学統計家の協力で本格的な関連解析　統計数理研の2先生に参加していただく
- ・関連性のある複数の多型の再現性を他のサンプルで確認

候補遺伝子アプローチによる関連多型の探索
- ・CYP (日本人で頻度5%以上のミスセンス変異: 例　106SNPsの解析を開始
- ・トランスポーター, 受容体などの関連遺伝子多型

遺伝子多型診断キットの作成
- ・GEANE研究で関連性の認められた複数の多型の中で特に関連性の強いものを載せたカスタムメードのマイクロアレイの作製
- ・このツールを用いた前向き臨床試験(GEANE2)の準備

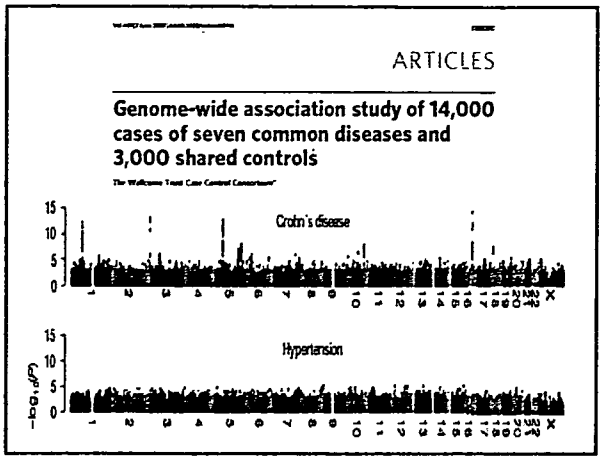
その他−遺伝子解析の簡便化(テーラーメード高血圧診療実現に向けての準備)
- ・FTAカードの試用可能性 (検証中)
  血液、口腔粘膜細胞を塗布する特殊な濾紙
  室温で、年単位の保存が可能

GEANE研究(降圧薬感受性遺伝子同定のための多施設前向き臨床試験)
- ゲノムワイドSNP遺伝子型決定チップの実際と検体集団の遺伝子的特性

国立循環器病センター研究所 循環分子生理部
花田 裕典
国立循環器病センター研究所 病因部
宮田 敏行、山本 勝広
国立循環器病センター研究所 臨床研究センター
富田 晃子
国立循環器病センター 高血圧腎臓内科
神出 計、河野 雄平

GEANE研究グループ

第30回 日本高血圧学会(沖縄)で発表予定
10/25 (木) 午後13:00～



次回会議(第8回)の日程

- 現在の候補日 <u>第1候補:</u>
  平成20年2月23日(土) 午後
  国立循環器病センター予定

  <u>第2候補:</u>
  平成20年2月16日(土) 午後

遺伝子解析の現状
（候補遺伝子解析）

国立循環器病センター研究所
腎臓高血圧内科／病因部
松本幸子

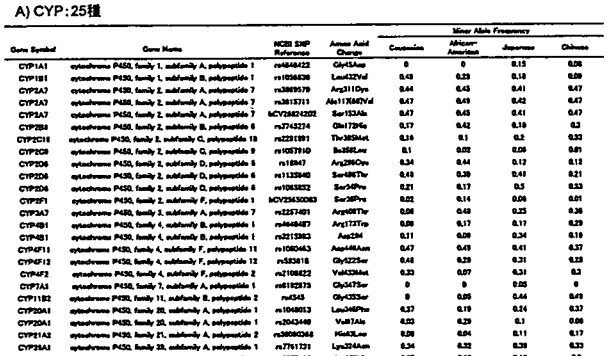
2008.2.16

対象と方法

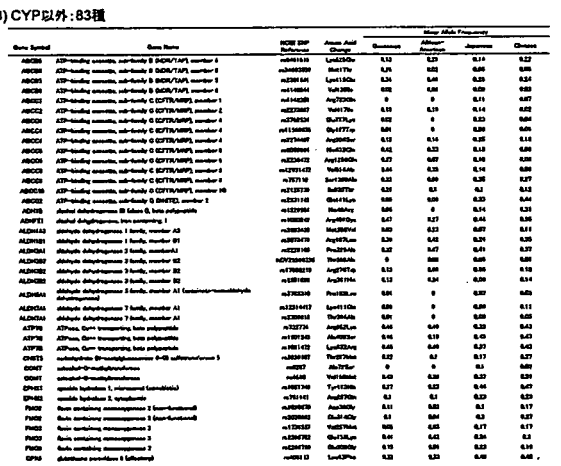
- GEANE研究　146検体
- Taqman Probe法にてSNPタイピング
- 候補遺伝子多型の選別
  1）薬物代謝／輸送関連遺伝子多型
  …ABI社Drug Metabolism Assaysより
  （ミスセンス変異、かつJPT-MAF≧0.05）
  2）既報の降圧剤感受性遺伝子多型

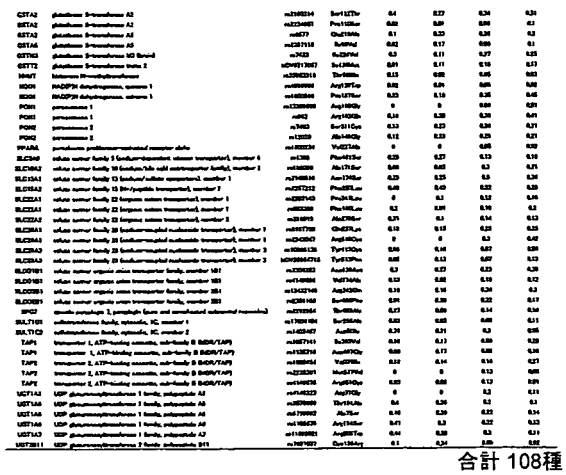
候補（1）薬物代謝／輸送関連遺伝子多型

A) CYP：25種



B) CYP以外：83種





合計 108種

候補（2）既報の降圧剤感受性遺伝子多型

合計 27種

Ⅱ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表（抜粋）

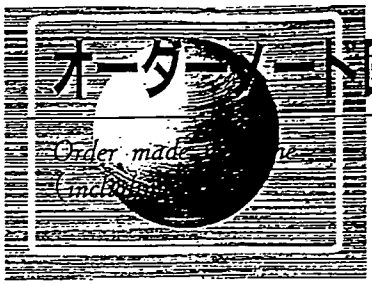
書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社	出版地	出版年	ページ
神出　計，河野雄平	オーダーメード医療(SNP を含む)	熊谷裕生，小室一成，堀内正嗣，森下竜一	高血圧ナビゲーター　第2版	メディカルレビュー社.	東京	2007	74-75

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kamide K, Kokubo Y, Fukuhara S, Hanada H, Yang J, Kada A, Nagura J, Takiuchi S, Horio T, Kawano Y, Okayama A, Tomoike H, Miyata T.	Protein tyrosine kinase 2 β as a candidate gene for hypertension.	Pharmacogenetics and Genomics	17(11)	931-939	2007
神出　計，宮田敏行，河野雄平	高血圧個別化診療に向けた臨床介入試験とゲノム解析の現況と展望	血管	28(3)	79-85	2005

Ⅲ. 研究成果の刊行物・別刷



オーダーメード医療(SNPを含む)

神出 計/河野雄平

高血圧診療において患者個人のもつ背景を考慮して治療方針を選択する必要があり，とくに遺伝素因から個人に合ったベストの治療をするオーダーメード医療の確立が望まれている．

オーダーメード医療とは

ポストゲノム時代の今，高血圧診療においても一塩基多型SNP(single nucleotide polymorphism；SNP)を解析することによって高血圧の発症や合併症を予測し，治療薬の選択を行うといった個人の遺伝子情報に基づいた診療，オーダーメード医療(テーラーメード医療，個別化医療)の確立に期待がかけられている．すでに癌やリウマチの治療においてはSNPを調べることで薬剤の副作用や効果を予測し，処方の際の情報とするオーダーメード医療が行われているが，高血圧診療においてはまだ研究段階で実現されていない．国立循環器病センターでは，オーダーメード医療の確立とゲノム創薬を目標に掲げた遺伝子解析計画であるミレニアム・ゲノム・プロジェクト(MGP)において高血圧を担当し，5年間で数多くの高血圧関連遺伝子を同定してきた[1]．MGPにより高血圧関連のゲノム情報の基盤は整備され，得られた膨大なゲノム情報はここ数年のうちに臨床の現場に応用されていくことは間違いないと考えられる．

オーダーメード医療への応用

1．高血圧原因遺伝子

高血圧への遺伝素因の関与は多岐にわたる．本態性高血圧(EHT)の病態の根幹をなすレニン-アンジオテンシン(RA)系や交感神経(SN)系の活性化，食塩感受性やインスリン抵抗性の形成など，すべての機序に遺伝因子は関与すると考えられる．また数多くの薬剤がこれらの病態をターゲットにして降圧作用を発揮する(図)．これまで数多く行われてきた候補遺伝子アプローチによる高血圧原因遺伝子同定の試みは，アンジオテンシン変換酵素(ACE)I/D多型に代表されるように，RA系やSN系の受容体や酵素の遺伝子をターゲットにして，ケース・コントロールを用いた解析が主流となってきた．さらに近年ではゲノム網羅的な方法で50万SNPをDNAマイクロアレイで調べる方法を用いて検討されているが[2]，糖尿病などと比較して非常に強い関連性をもつ高血圧原因遺伝子多型がいまだに同定できていないのが現状である．倫理問題を含め，高血圧原因遺伝子多型を調べることで将来の高血圧発症を予測することは当面難しいと思われる．

2．高血圧性臓器障害関連遺伝子

高血圧患者の予後を左右するのは心・血管・腎の合併症であり，遺伝子情報を用いたオーダーメード医療により遺伝子多型から合併症の進展が予測できれば，より厳格な降圧を試みたり，それぞれの臓器障害に有効とされる薬剤を早期から服薬させることにより，発症を予防することが可能となると考えられる．

3．降圧薬感受性遺伝子

降圧薬服用者は高血圧治療患者の半数以上を占める．したがって遺伝的に規定されている降圧薬に対する感受性を薬剤選択の際に考慮できれば，効率のよい降圧薬治療を実現することが可能となる．

用語解説———DNAマイクロアレイ(チップ)
種々のDNAをスライドグラスもしくはシリコンの基盤に非常に繊細に並べたもので，ハイブリダイゼーション法によりDNAの多型を一度に大量に解析できるもの．最近では50〜100万個のSNPを解析するチップが出ている．

Recommended Readings
❶ Turner ST et al : J Hypertens 19 : 1-11, 2001
❷ Arnett DK et al : Circulation 115 : 2878-2901, 2007

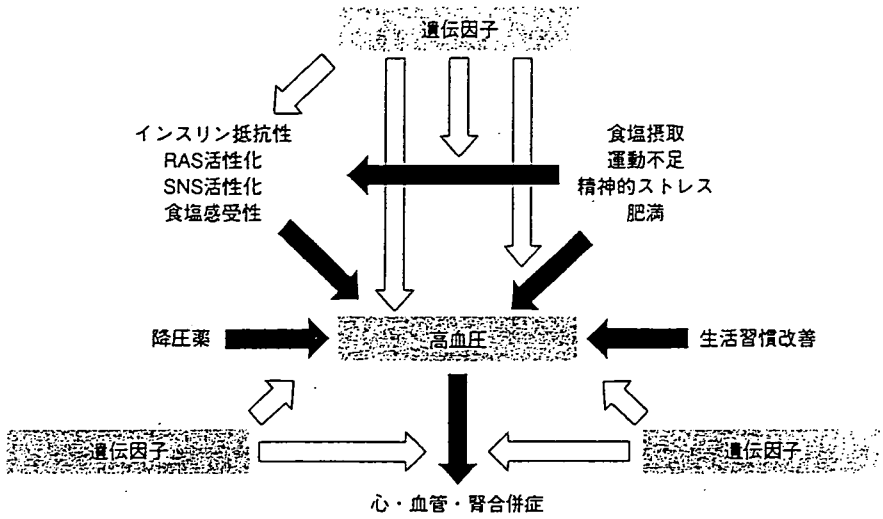


図　高血圧における遺伝因子の影響
(Kamide K et al : Jpn Heart J 45 : S69, 2004 より引用)

こういった観点から降圧薬感受性遺伝子多型を明らかにするために，近年 pharmacogenomics なアプローチがなされてきた．しかしながら，多くの研究が RA 系や SN 系の酵素や受容体の一つから数個の多型の関与を調べた検討のみで，結果は非常にコントロバーシャルであった[3]．より関連性の強い薬剤応答性・感受性遺伝子の同定のためには，多数例の無治療高血圧患者に前向きに降圧薬を投与し，正確に降圧の程度を把握し，数多くの薬物代謝酵素や薬理作用機序関連の遺伝子多型との相関を検討する必要がある．これまでわが国にこのような研究はなかったが，現在，国立循環器病センターでは，全国の大学・医療センターなどとともに降圧薬感受性遺伝子多型同定のための多施設共同研究(GEANE 研究)を施行している．GEANE 研究では，無投薬の軽・中等症 EHT 患者にサイアザイド系利尿薬，ARB，Ca 拮抗薬を 3 カ月ごとに内服してもらい，観察期も含め合計 10 カ月間で投薬を終了するデザインで施行中である．降圧効果のみならず，副作用や代謝性の異常も解析予定でゲノム網羅的に複数の SNP を検討し，これら 3 種類の薬剤の感受性遺伝子多型ならびに副作用関連遺伝子多型を検索している．これにより同定された遺伝子多型を実際の臨床に応用し，オーダーメード医療を確立することを構想している．

オーダーメード医療の実現に向けて

高血圧のオーダーメード医療実現には，適確な研究成果の集積と，出てきた遺伝子多型を用いた迅速遺伝子診断システムの開発，このような遺伝子診断システムを導入した場合の有用性を確かめる前向き試験，遺伝子診断を考慮した新しい高血圧診療ガイドラインの制定などが必要と考えられ，道程は長い．しかしながら確実な研究成果の集積により必ずや実現できるであろう．無駄が少なく，より安全で，合併症を減少させることができるような高血圧診療を患者に提供することを最終目標に研究を進めることが重要である．

References
1) 神出　計 ほか：血管 28：79-85, 2005
2) The Wellcome Trust Case Control Consortium : Nature 447 : 661-678, 2007
3) Arnett DK et al : Circulation 111 : 3374-3383, 2005

関連事項
吹田研究 ▶▶ 50 頁
ACE 多型 ▶▶ 62 頁
ミレニアム・プロジェクト ▶▶ 72 頁
高血圧の遺伝因子 ▶▶ 150 頁

Protein tyrosine kinase 2β as a candidate gene for hypertension

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Protein tyrosine kinase 2β (PTK2B) is a member of the focal adhesion kinase family and is activated by angiotensin II through Ca^{2+}-dependent pathways. An evidence exists that PTK2B is involved in cell growth, vascular contraction, inflammatory responses, and salt and water retention through activation of the angiotensin II type 1 receptor. To examine the contribution of PTK2B, we sequenced the *PTK2B* gene using 48 patients with hypertension, identified 62 genetic polymorphisms, and genotyped six representative single nucleotide polymorphisms in population-based case–control samples from 3655 Japanese individuals (1520 patients with hypertension and 2135 controls). Multivariate logistic regression analysis after adjustments for age, body mass index, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) showed −22A>G to have an association with hypertension in men (AA vs. AG+GG: odds ratio=1.27; 95% confidence interval: 1.02–1.57; *P*=0.030). Another polymorphism, 53484A>C (K838T), in linkage disequilibrium with −22A>G showed a marginal association with hypertension in men (AA vs. AC+CC: odds ratio=1.25; 95% confidence interval: 0.99–1.57; *P*=0.059). Diastolic blood pressure was 1.6 mmHg higher in men with the AC+CC genotype of 53484A>C than those with the AA genotype (*P*=0.003), after adjustments for the same factors. These polymorphisms are in linkage disequilibrium with others in a range of 113 kb in *PTK2B*. The intracellular distribution of the recombinant PTK2B protein and that of the mutant protein with T838 were indistinguishable even after angiotensin II stimulation, both proteins localizing at a focal point in the peripheral area in the cells. Thus, a haplotype in *PTK2B* may play a role in essential hypertension in Japanese. *Pharmacogenetics and Genomics* 17:931–939

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Introduction

Angiotensin II (Ang II) is a multifunctional hormone that regulates the functions of cardiovascular cells through intracellular signaling events initiated via interaction with cell surface Ang II type 1 (AT1) and Ang II type 2 (AT2) receptors [1,2]. Ang II was initially described as a vasoconstrictor. Recent studies, however, demonstrate that Ang II has growth factor and cytokine-like properties. The multiple actions of Ang II are mediated by specific intracellular signaling pathways that are stimulated following initial binding of the peptide to its specific receptors. In the vasculature, AT1 receptors are mainly present in vascular smooth muscle cells (VSMCs) [3]. In the heart, AT1 receptors are expressed in cardiomyocytes and fibroblasts. AT1 receptors mediate most of the physiological actions of Ang II.

Protein-tyrosine kinase 2β (PTK2B), also known as proline-rich tyrosine kinase-2, focal adhesion kinase 2 (FAK2), cell-associated kinase β, related focal tyrosine kinase or calcium-dependent tyrosine kinase, exhibits a considerable level of structural homology to FAK, a nonreceptor tyrosine kinase which targets sites of integrin clustering [4]. Unlike FAK, PTK2B is expressed in a highly cell type and tissue-specific manner. PTK2B is activated by phosphorylation on tyrosine residues in response to various stimuli, depending on the cell type, including G protein-coupled receptor agonists (such as Ang II, thrombin, and lysophosphatidylcholine) and cellular stress (from ultraviolet irradiation, tumor necrosis factor-α, hyperosmotic shock, etc.) [5]. Activation of PTK2B requires intracellular calcium release [6]. In contrast to FAK, which is localized to adhesion plaques at the basal side of the cell, PTK2B is localized in the cytosol but can be recruited to plasma membrane, the perinuclear region, or the nucleus in response to different stimuli [7].

PTK2B is very similar to FAK, containing a kinase domain and two proline-rich domains, as well as several phosphorylated residues including an autophosphorylation site (T402), sites involved in kinase activation (T579, T580),

and a site (T881) homologous to the Grb2-binding site in FAK [6,8].

AT1 receptors activate PTK2B in a calcium-dependent manner. As PTK2B may act to regulate c-Src and to link G protein-coupled vasoconstrictor receptors with protein kinase-mediated contractile, migratory, and growth responses, it may be a potential point of convergence between Ca^{2+}-dependent signaling pathways and protein kinase pathways in VSMCs. Thus, PTK2B may play a role in hypertension through AT1 receptors.

In this study, we attempted to evaluate the *PTK2B* gene in relation to hypertension using population-based case–control samples from 3655 Japanese individuals (1520 patients with hypertension and 2135 controls). First we identified genetic variations, mainly single nucleotide polymorphisms (SNPs), in all exons of *PTK2B*. Next, we examined the association of SNPs with the presence of hypertension in the Japanese general population. Finally, we examined the intracellular localization of a mutant PTK2B with the missense mutation K838T.

Methods

Participants of the study population

The selection criteria and design of the Suita study were described previously [9,10]. Briefly, the participants had been selected randomly from the municipal population registry and stratified based on sex and age (stratified in 10-year intervals). They were all invited, by letter, to receive medical and behavioral examinations every 2 years at the Division of Preventive Cardiology, National Cardiovascular Center, Japan. DNA from the leukocytes was collected from participants who visited the National Cardiovascular Center. In this study, 3655 individuals including 1520 patients with hypertension (779 men, 741 women) and 2135 controls (930 men, 1205 women) were genotyped. All of the participants were Japanese. For DNA sequencing, 48 Japanese patients with essential hypertension at the Division of Hypertension and Nephrology, National Cardiovascular Center, Japan, were recruited. Only those who gave their written informed consent for genetic analyses were included in this study. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

Measurements

Blood pressure was measured after at least 10 min of rest in a sitting position. Systolic and diastolic blood pressures (SBP and DBP) were the means of two measurements (recorded > 3 min apart). In this study, two criteria were used to define hypertension. SBP of ≥ 140 mmHg and/or DBP of ≥ 90 mmHg, or the current use of antihypertensive medication. To exclude marginal hypertension, hypertension was defined as SBP of ≥ 160 mmHg and/or DBP of ≥ 95 mmHg, or the current use of antihypertensive medication. Diabetes mellitus was defined as a fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose level ≥ 11.1 mmol/l (200 mg/dl) or the taking of antidiabetic medication or HbA1c ≥ 6.5%. Hyperlipidemia was defined as a total cholesterol concentration ≥ 5.68 mmol/l (220 mg/dl) or the taking of antihyperlipidemia medication. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Blood samples drawn from the participants after 12 h of fasting were collected in ethylenediaminetetraacetate-containing tubes. Total cholesterol and high-density lipoprotein cholesterol levels were measured with an autoanalyzer (Toshiba TBA-80, Tokyo, Japan) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Health Science and Promotion, Japan.

Direct sequencing for discovering polymorphisms and genotyping of single nucleotide polymorphisms

We sequenced the entire coding region of *PTK2B* in 48 hypertensive samples in which hypertension susceptive polymorphisms would be much concentrated. The methods of direct sequencing were described previously [11,12]. SNPs having a minor allele frequency of greater than 5% were candidates for genotyping using the TaqMan-polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, California, USA) [13,14]. As a consequence, we genotyped six SNPs in the population-based samples.

Linkage disequilibrium and single nucleotide polymorphism blocks in the *PTK2B* gene

SNP genotype data for the Japanese population were downloaded from the International HapMap Consortium (*www.hapmap.org*) [15]. Positions of SNP sites were renumbered by NCBI human chromosome sequences (build 35). Pair-wise *D'* and LOD values were calculated and SNP blocks were inferred by Haploview [16] using its default setting parameters.

Expression of wild-type and mutant rat PTK2B

PTK2B is a rat ortholog of human PTK2B. PTK2B cDNA was inserted into an EGFP-tagging mammalian expression vector, pEGFP-C1 (BD Biosciences, San Jose, California, USA). A missense mutation, K838T, was introduced into PTK2BcDNA by site-directed mutagenesis using PCR. The mutation was confirmed by sequencing. Human umbilical vascular endothelial cells (HUVECs) were cultured in HuMedia-2 (Kurabo, Osaka, Japan) supplemented with a growth additive set and used for experiments before passage 7. HUVECs cultured on glass-bottom dishes transfected with either pEGFP-wild-type PTK2B or pEGFP-mutant PTK2B using FuGene6 (Roche Diagnostics, Basel, Switzerland) were imaged under a fluorescence microscope (IX-81, Olympus,

Tokyo, Japan). Furthermore, both wild-type and mutant cells were incubated with vehicle or 1 μmol/l Ang II (Sigma, St Louis, Missouri, USA) for 5 min to investigate the difference of cell maturity.

Statistical analysis

Student's *t*-test was used to compare mean values between groups. Frequencies were compared by χ^2 analysis. The relationships in men and women between genotypes and the presence of hypertensives were expressed in terms of odds ratios (ORs) adjusted for possible confounding effects including age, BMI, antihypertensive drug use, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) by logistic regression analysis. For multivariate risk predictors, adjusted ORs were given with 95% confidence intervals (CIs).

Association-based analyses in each sex of genotypes with blood pressures were investigated through analysis of covariance considering potential confounding risk variables, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), lifestyle (smoking and drinking), and antihypertensive medication.

Statistical analyses were performed with SAS statistical software (release 6.12, SAS Institute Inc., Cary, North Carolina, USA). The linkage disequilibrium (LD) of genotyped SNPs was calculated by using SNPAlyze version 2.1 (DYNACOM Co., Ltd, Mohara, Japan).

Results

Basic characteristics of participants of the study population

The characteristics of the 3655 participants (1709 men, 1946 women) are summarized in Table 1a. Age, SBP, DBP, BMI, percentage that are current smokers, percentage that are current drinkers, prevalence of hypertension, and prevalence of diabetes mellitus were significantly higher in men than in women. Total cholesterol, high-density lipoprotein cholesterol, and percentage that have hyperlipidemia were significantly higher in women than in men. Table 1b shows patients characteristics divided by two criteria of hypertension. Age, BMI, and percentage that are current drinkers, have diabetes and have hyperlipidemia were higher in hypertensive patients than normotensives for both criteria.

Polymorphisms in *PTK2B* and genotyping of single nucleotide polymorphisms

We sequenced 96 alleles from 48 Japanese patients with hypertension, and identified 62 polymorphisms, including four nonsynonymous and 11 synonymous SNPs (Table 2). The four nonsynonymous SNPs, 45344G > A, 48255A > G, 48273G > A, and 53484A > C, encode for the missense mutation R698H with a minor allele frequency of 0.010,

Table 1a Basic characteristics of the participants

	Women (n=1946)	Men (n=1709)
Age (year)	63.5±11.1	66.1±11.3*
Systolic blood pressure (mmHg)	128.3±19.8	130.8±19.1*
Diastolic blood pressure (mmHg)	76.5±9.7	79.2±10.3*
Body mass index (kg/m^2)	22.4±3.2	23.3±3.0*
Total cholesterol (mg/dl)	216.1±31.3*	198.7±31.4
HDL cholesterol (mg/dl)	64.9±15.2*	54.9±14.3
Current smokers (%)	6.0	30.0†
Current drinkers (%)	27.3	66.9†
Present illness (%)		
Hypertension	38.1	45.6†
Hyperlipidemia	54.9†	31.5
Diabetes mellitus	6.1	13.0†

Values are the mean±SD or a percentage. Hypertension indicates a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or antihypertensive medication; hyperlipidemia, total cholesterol ≥ 5.68 mmol/l (220 mg/dl) or antihyperlipidemia medication; diabetes, fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose ≥ 11.1 mmol/l (200 mg/dl) or antidiabetic medication. HDL, high-density lipoprotein.
*P<0.05 between women and men by the Student's *t*-test.
†P<0.05 between women and men by the χ^2 test.

Table 1b Characteristics of the patients divided by two definitions of hypertension

	NT1 (n=2135)	HT1 (n=1520)	NT2 (n=2557)	HT2 (n=1098)
Age (year)	61.8±11.6	68.8±9.4*	62.7±11.4	69.5±9.4*
Sex (F/M)	1205/930	741/779+	1426/1131	520/578+
Body mass index (kg/m^2)	22.3±3.0	23.6±3.2*	22.4±3.0	23.8±3.2*
Systolic blood pressure (mmHg)	118.0±12.0	145.6±16.5*	122.6±15.3	145.5±18.9*
Diastolic blood pressure (mmHg)	73.9±8.3	83.2±9.8*	75.6±9.0	82.9±10.5*
Total cholesterol (mg/dl)	208.3±32.8	207.5±32.2	208.7±32.5*	206.2±32.5
HDL cholesterol (mg/dl)	61.3±15.8*	58.7±15.2	61.1±15.8*	58.1±14.9
Current smokers (%)	19.3+	14.2	18.9+	13.4
Current drinkers (%)	43.6	49.0+	44.3	49.3+
Present illness (%)				
Diabetes mellitus	6.9	12.8+	7.4	13.8+
Hyperlipidemia	40.6	48.6+	41.8	49.1+

Values are the mean±SD or a percentage. HDL, high-density lipoprotein; HT, hypertension; HT1, hypertension indicates a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or antihypertensive medication; HT2, hypertension indicates a systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg or antihypertensive medication; hyperlipidemia, total cholesterol ≥ 5.68 mmol/l (220 mg/dl) or antihyperlipidemia medication; diabetes mellitus, fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose ≥ 11.1 mmol/l (200 mg/dl) or antidiabetic medication; NT, normotension.
*P<0.05 between cases and controls by the Student's *t*-test.
+P<0.05 between cases and controls by the χ^2 test.

Table 2 List of polymorphisms and their allele frequency in *PTK2B* identified by direct sequencing in 48 hypertensive patients

SNP	LD	Amino acid substitution	Region	Allele 1 frequency	Allele 2 frequency	Flanking sequence	Typing	dbSNP ID
-86282C>A			Promoter	0.870	0.130	cccggctgccaa[c/a]gcccgcgacccg	Taqman	rs7006183
-86255C>T	a		Promoter	0.924	0.076	tgctgggaatcg[c/t]ccagtcccttcc		rs12679503
-86253C>T			Promoter	0.989	0.011	ctgggaatcgcc[c/t]agtcccttcccc		
-86200G>A	b		Promoter	0.967	0.033	caatcgtgcggg[g/a]gggatggcgagg		
-86188G>A	b		Promoter	0.967	0.033	ggggatggcgag[g/a]gggagg-gagggg		
-86141G>A	c		Promoter	0.761	0.239	cttccggtgtgc[g/a]cgggaaatcttg	Taqman	rs7005244
-85972A>T	c		5′-UTR	0.771	0.229	AAAGGAGCCTCT[A/T] CCTTAACCAATC		rs6988218
-85868C>T	a		Intron 1	0.927	0.073	GCACCgtgagtg[c/t]aatcaccactta		rs12679570
-75144G>A	d		5′-UTR	0.979	0.021	TTGTGAAGACAA[G/A] CTAGACGGCAGA		
-74037T>C	c		Intron 5	0.719	0.281	cattattgcaac[t/c]tatgccatatgg		
-99G>A	e,g,i,k,m		Intron 6	0.589	0.411	gctgtccctggg[g/a]ccatgaggtatg		
-22A>G	e,g,i,k,m		5′-UTR	0.589	0.411	TGCAATGTGCCG[A/G] TCTTAGCTGCTG	Taqman	rs2241649
27T>C	e,g,i,k,m	S9S	Exon 7	0.589	0.411	CGAGCCCCTGAG[T/C] CGAGTAAAGTTG		rs1045510
45G>A	e,g,i,k,m	T15T	Exon 7	0.589	0.411	AAAGTTGGGCAC[G/A] TTACGCCGGCCT		rs1045511
162A>G	e,g,i,k,m	K54K	Exon 7	0.589	0.411	CAATCCTGGGAA[A/G] AACTTCAAACTG		rs1045512
224C>T	e,g,i,k,m		Intron 7	0.589	0.411	tgaagtgtctgc[c/t]ctgtccatctgt		rs2241650
22313G>A		E63E	Exon 8	0.990	0.010	tcctctgcagGA[g/a] ATCATCACCTCC		
22436G>A	f	T110T	Exon 8	0.875	0.125	CCCACAGATGAC[G/A] GTGGGTGAGGTG	Taqman	rs1030526
24604G>A	e,g,h,k,l		Intron 9	0.427	0.573	ttgtttgtggtg[g/a]ggtgggtggctg		rs2241652
32896T>A			Intron 12	0.865	0.135	gagtgtaaggga[t/a]gaggctggggct	Taqman	rs2241653
32932T>C	f		Intron 12	0.885	0.115	gagaagccaggg[t/c]atctgcgcggcc		rs7827965
33213T>C			Intron 12	0.927	0.073	agcagtgggcag[t/c]ctctcagcgaga		
33938C>T			Intron 14	0.957	0.043	gggaggtcgtcc[c/t]ctctctgctgcc		
34834T>C			Intron 15	0.979	0.021	tataatggcaga[t/c]tgggagctcttg		rs2303881
34862T>C			Intron 15	0.979	0.021	agaccaaaagtc[t/c]gtgacacacagg		rs2303882
36097G>A			Intron 16	0.979	0.021	ccacagcccagc[g/a]ggaagcttccag		
36456T>C	g,h,k,l		Intron 16	0.417	0.583	gtcagtcaccca[t/c]ccaggtccctgt		rs919493
36567A>G			Intron 17	0.979	0.021	acaatggggtgc[a/g]gaggacagggcc		
36648C>T	b		Intron 17	0.948	0.052	catagtttctgg[c/t]tttcaggcccag		rs12056620
38234G>T	e,i,j,k,m		Intron19	0.615	0.385	ccccgccacagc[g/t]accgtagtcaag		rs11774417
38312C>T			Intron 19	0.989	0.011	ttcctcctttat[c/t]ctcccttcgtgc		
38764C>T	b	H447H	Exon 20	0.958	0.042	CTACACAAATCA[C/T] gtgagttctagg		rs7005936
38881C>T			Intron 20	0.990	0.010	gggccccttgtc[c/t]ctaagggcctct		
38888G>A	b		Intron 20	0.958	0.042	ttgtccctaagg[g/a]cctcttgtccac		rs2241654
39431C>G	g,h,k,l		Intron 20	0.426	0.574	taggagaaaggg[c/g]cctttcctggca		rs2163176
39505G>C	b		Intron 20	0.957	0.043	agcactgggctg[g/c]accaaggggtcc		rs7005954
39722T>C	g,h,k,l		Intron 21	0.426	0.574	tggaggaggggt[t/c]cccgtcctccca		rs6996922
41359-41360delTC			Intron 23	0.990	0.010	agattcttggtc[tc/-]tttttccatctg		
42101T>C	b		Intron 25	0.958	0.042	aagacgaaactc[t/c]gtgacttattct		rs11995441
42595T>C	g,h,k,l		Intron 25	0.448	0.552	ggcgatggtgct[t/c]ctgggtgggagg		rs2241657
42977A>G	g,h,k,l		Intron 26	0.448	0.552	agggtcaaggac[a/g]ggaggctgaagc		rs2241658
45344G>A		R698H	Exon 27	0.990	0.010	AGAGGAATGCTC[G/A] CTACCGAACCCC		
46624C>G	i,j	P717P	Exon 28	0.755	0.245	ctctctccagCC[C/G] AGCCGACCTAAG		
48255A>G		M754V	Exon 29	0.989	0.011	CTCACCAGCCCT[A/G] TGGAGTATCCAT		
48273G>A	d	V760F	Exon 29	0.979	0.021	TATCCATCTCCC[G/A] TTAACTCACTGC		
48640T>A			Intron 29	0.989	0.011	ggggtaggggga[t/a]ctgtggcagctt		
53437G>A	b		Intron 30	0.957	0.043	tcttagtccttc[g/a]ctcttgtttctt		rs751018
53443G>A			Intron 30	0.989	0.011	tccttcgctctt[g/a]tttcttcctctg		
53484A>C	e,g,h,i,k,l,m	K838T	Exon 31	0.489	0.511	ATATGAATGATA[A/C] GTCCCCATTGgt	Taqman	rs751019
53748A>G	g,h,k,l,m		Intron 31	0.448	0.552	cagaaaggtcac[a/g]ttgggtcacgag		rs2251430
53860C>T	e,i,k,l,m		Intron 31	0.615	0.385	tgtctccacagc[c/t]gcatgagtgacg		rs2278319
55445A>G	g,h,k,l		Intron 32	0.448	0.552	tggtagagggga[a/g]ggggctcatttg		rs3735758
56602T>C	g,h,k,l	T876T	Exon 34	0.448	0.552	CCTGGACCGGAC[T/C] GATGACCTGGTG		rs1879184
56804-56805delCT			Intron 34	0.990	0.010	ccagcagatcct[ct/-]tagagcaagctg		
56939C>T	n		Intron 34	0.956	0.044	ctgccccttttct[c/t]cccccagAATGT		rs10093964
57034G>A	n		Intron 35	0.956	0.044	ACAGAGgtgagc[g/a]tcccattccaga		rs7007145

Table 2 (*continued*)

SNP	LD	Amino acid substitution	Region	Allele 1 frequency	Allele 2 frequency	Flanking sequence	Typing	dbSNP ID
60775A>G	g,h,k,l	A960A	Exon 36	0.435	0.565	GATGCGGCTGGC[A/G] CAGCAGAACGCC		rs1879182
60799A>G	g,h,k,l	L968L	Exon 36	0.435	0.565	CGTGACCTCCCT[A/G] AGTGAGGAGTGC		rs1879181
60835A>G		S980S	Exon 36	0.967	0.033	GCTGACGGCTTC[A/G] CACACCCTGGCT		
60926C>T			3′UTR	0.989	0.011	CCTGCAGAGTGA[C/T] GGAGGGTGGGGG		
61000T>C			3′UTR	0.989	0.011	TGCTGTTGGTCA[T/C] GTGGGTCTTCCA		
61016G>A	b		3′UTR	0.957	0.043	GGTCTTCCAGGG[G/A] GAAGGCCAAGGG		rs2271920

The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (*Hum Mut* 1998; 11:1–3). The uppercase and lowercase letters are the sequence in the exon and intron region, respectively. The nucleotide sequence (GenBank Accession ID: NT_023666.16) was used as a reference sequence. The apparent linkage disequilibrium (LD), defined by an r^2 of more than 0.5, was indicated by a–m, which shows different LD group.
SNP, single nucleotide polymorphism; UTR, untranslated region; Taqman, the SNP was successfully genotyped by the Taqman method.

for M754V with a minor allele frequency of 0.011, for V760F with a minor allele frequency of 0.021, and for K838T with a minor allele frequency of 0.489, respectively. 53484A > C has been deposited in the public database with the dbSNP number, rs751019. Considering the allele frequency and the LD, we selected six SNPs for genotyping in large-scale population-based samples.

Association of single nucleotide polymorphisms with hypertension

The multivariate logistic regression analysis after adjustments for age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) revealed that −22A > G showed an association with the presence of hypertension in men (AA vs. AG + GG: OR = 1.27; 95% CI: 1.02–1.57; $P = 0.030$). Another polymorphism, 53484A > C, accompanied by the missense mutation K838T in LD with −22A > G, showed a marginal association with the presence of hypertension in men (AA vs. AC + CC: OR = 1.25; 95% CI: 0.99–1.57; $P = 0.059$) (Table 3a). Power analyses using the SNPs with hypertension were performed. These two significant SNPs, −22A > G and 53484A > C (K838T), showed higher power, 68 and 53%, respectively. Furthermore, DBP was 1.6 mmHg higher in men with the AC + CC genotype of 53484A > C than those with the AA genotype ($P = 0.003$), after adjustments for the same factors described above (Table 4).

When hypertension was defined as SBP of > 160 mmHg and/or DBP of > 95 mmHg, or the current use of antihypertensive medication, −22A > G showed an association with the presence of hypertension in men (AA vs. AG + GG: OR = 1.38; 95% CI: 1.10–1.73; $P = 0.006$). Another polymorphism, 53484A > C (K838T), showed a significant association with the presence of hypertension in men (AA vs. AC + CC: OR = 1.31; 95% CI: 1.03–1.68; $P = 0.031$) (Table 3b). These two significant SNPs, −22A > G and 53484A > C (K838T), showed power, 90 and 68%. Taken together, *PTK2B* was associated with the presence of hypertension in men.

The pair-wise LD parameters, r^2 and D', calculated from the genotype data for these SNPs, are shown in Table 5. Two SNPs, −22A > G (rs1045510) and 53484A > C (K838T: rs751019), were in LD with an r^2 of more than 0.5. These polymorphisms showed LD extensively to make a haplotype block with more than 20 SNPs, as shown in Table 2.

To understand more about the LD in *PTK2B*, we retrieved genotype data on *PTK2B* from the public database, HapMap Project. The pair-wise D' value is shown in supplement Fig. 1. A hypertension-associated polymorphism, 53484A > C (K838T: rs751019), was in LD with rs1879181, rs1583092, rs1019832, rs4733058, rs725787, rs2322718, rs1045510, rs919495, rs11776858, rs11785606, rs10109834, and rs3735759, which are present in a stretch of 113 kb in *PTK2B*. Among the polymorphisms in LD with 53484A > C (K838T: rs751019), rs1045510 (27T > C) is a synonymous SNP encoding S9S, and the others are present in the 5′-untranslational region, in an intron, or in the 3′-untranslational region.

Expression of mutant PTK2B

As described, a haplotype of *PTK2B* including the missense mutation K838T was associated with the presence of hypertension. Figure 1 shows an amino acid sequence alignment of human, ape, dog, and mouse, in the area surrounding K838. The amino acid sequence around K838 was highly conserved among mammals, suggesting a functional role. To understand the functional roles of the K838T mutation, the rat ortholog, PTK2B, was expressed in HUVECs to see the effects on the intracellular localization of the recombinant protein. Both EGFP-tagged wild-type PTK2B and mutant PTK2B were

Table 3a Allele frequency and odds ratio of the presence of hypertension by genotypes of *PTK2B* polymorphisms by sex

		Men		Women	
SNP (allele frequency)	Genotype group	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
-86282C>A	CC	1	0.427	1	0.074
(0.890/0.110)	CA+AA	0.90 (0.70–1.16)		1.26 (0.98–1.62)	
	CC+CA	1	0.914	1	0.181
	AA	1.05 (0.45–2.45)		1.78 (0.76–4.16)	
-86141G>A	GG	1	0.588	1	0.428
(0.671/0.329)	GA+AA	1.06 (0.86–1.30)		1.09 (0.89–1.33)	
	GG+GA	1	0.571	1	0.722
	AA	1.10 (0.79–1.55)		0.94 (0.67–1.32)	
-22A>G	AA	1	0.030	1	0.521
(0.597/0.403)	AG+GG	1.27 (1.02–1.57)		1.07 (0.87–1.32)	
	AA+AG	1	0.375	1	0.617
	GG	1.14 (0.86–1.50)		0.93 (0.70–1.24)	
22436G>A	GG	1	0.456	1	0.452
(0.820/0.180)	GA+AA	0.92 (0.74–1.14)		0.92 (0.74–1.14)	
	GG+GA	1	0.483	1	0.764
	AA	1.27 (0.65–2.45)		1.10 (0.58–2.09)	
32896T>A	TT	1	0.935	1	0.254
(0.889/0.111)	TA+AA	1.01 (0.79–1.29)		1.15 (0.90–1.48)	
	TT+TA	1	0.276	1	0.926
	AA	1.59 (0.69–3.67)		0.96 (0.37–2.50)	
53484A>C	AA	1	0.059	1	0.874
K838T	AC+CC	1.25 (0.99–1.57)		0.98 (0.79–1.22)	
(0.527/0.473)	AA+AC	1	0.600	1	0.633
	CC	1.07 (0.83–1.37)		0.94 (0.73–1.21)	

All adjusted for age, body mass index, antihypertensive drug use, present illness (hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking). Hypertension was defined as a systolic blood pressure of >140 mmHg and/or diastolic blood pressure of >90 mmHg, or the current use of antihypertensive medication.
CI, confidence interval; SNP, single nucleotide polymorphism.

Table 3b Allele frequency and odds ratio of the presence of hypertension by genotypes of *PTK2B* polymorphisms by sex

		Men		Women	
SNP (allele frequency)	Genotype group	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
-86282C>A	CC	1	0.426	1	0.288
(0.890/0.110)	CA+AA	1.11 (0.86–1.44)		1.16 (0.88–1.52)	
	CC+CA	1	0.881	1	0.657
	AA	1.07 (0.45–2.51)		1.24 (0.48–3.15)	
-86141G>A	GG	1	0.940	1	0.100
(0.671/0.329)	GA+AA	1.01 (0.81–1.25)		1.20 (0.96–1.50)	
	GG+GA	1	0.470	1	0.305
	AA	1.14 (0.80–1.60)		0.82 (0.57–1.19)	
-22A>G	AA	1	0.006	1	0.819
(0.597/0.403)	AG+GG	1.38 (1.10–1.73)		1.03 (0.82–1.29)	
	AA+AG	1	0.067	1	0.985
	GG	1.31 (0.98–1.74)		1.00 (0.74–1.37)	
22436G>A	GG	1	0.060	1	0.490
(0.820/0.180)	GA+AA	0.80 (0.64–1.01)		0.92 (0.73–1.16)	
	GG+GA	1	0.735	1	0.360
	AA	1.13 (0.56–2.26)		1.36 (0.70–2.63)	
32896T>A	TT	1	0.420	1	0.475
(0.889/0.111)	TA+AA	1.11 (0.86–1.43)		1.10 (0.84–1.44)	
	TT+TA	1	0.691	1	0.487
	AA	1.18 (0.52–2.68)		1.42 (0.53–3.84)	
53484A>C	AA	1	0.031	1	0.554
K838T	AC+CC	1.31 (1.03–1.68)		1.07 (0.85–1.36)	
(0.527/0.473)	AA+AC	1	0.986	1	0.386
	CC	1.00 (0.77–1.30)		0.89 (0.67–1.16)	

CI, confidence interval; SNP, single nucleotide polymorphism.
All adjusted for age, body mass index, antihypertensive drug use, present illness (hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking). Hypertension was defined as a blood pressure of >160 mmHg and/or diastolic blood pressure of >95 mmHg, or the current use of antihypertensive medication.

observed at the focal adhesions. Figure 2a and b indicated that the missense mutation, K838T, of PTK2B does not extensively alter the intracellular localization of PTK2B. As shown in Fig. 2c and d, both EGFP-tagged wild-type PTK2B and mutant PTK2B were observed at the cytosol and the immature focal adhesions without the stimulation. After Ang II stimulation, EGFP-tagged wild-type PTK2B and mutant PTK2B were partially located at the mature focal adhesions as reported previously [17] and had similar localization manner. These results indicated that the missense mutation, K838T, of PTK2B does not extensively alter the intracellular localization of PTK2B.

Table 4 Blood pressure levels by genotypes of a *PTK2B* polymorphism, 53484A>C (K838T), in men

	AA	AC	CC	*P*	AA+AC	CC	*P*	AA	AC+CC	*P*
n	459	876	366		1335	366		459	1242	
DBP	78.0±0.5	79.9±0.3	78.9±0.5	0.123	79.2±0.3	78.9±0.5	0.544	78.0±0.5	79.6±0.3	0.003
SBP	130.8±0.8	131.0±0.6	130.5±0.9	0.882	130.9±0.5	130.5±0.9	0.720	130.8±0.8	130.8±0.5	0.921

Values are mean±SD. All adjusted for age, body mass index, antihypertensive drug use, present illness (hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking).
DBP, diastolic blood pressure; SBP, systolic blood pressure.
DBP and SBP are expressed in mmHg.

Table 5 Linkage disequilibrium of six genotyped *PTK2B* polymorphisms expressed by r^2 and absolute D'

	−86282 C>A	−86141 G>A	−22 A>G	22436 G>A	32896 T>A	53484 A>C
−86282C>A	–	0.24	0.17	0.02	0.41	0.09
−86141G>A	1.00	–	0.14	0.07	0.07	0.04
−22A>G	0.94	0.42	–	0.13	0.09	0.53
22436G>A	0.79	0.40	0.97	–	0.02	0.02
32896T>A	0.66	0.50	0.68	0.89	–	0.13
53484A>C	0.82	0.27	0.85	0.36	0.94	–

Upper right represents r^2 and lower left shows absolute D'.

Fig. 1

		*	
Human	830	DPMVYMNDKSPLTPEKEV	847
Ape	923	DPMVYMNDKSPLTPEKEV	940
Dog	830	DPMLYMNDKSPLTPEKEA	847
Mouse	830	DPMVYMNDKSPLTPEKEA	847
Rat	788	DPMVYMNDKSPLTPEKEA	805

Amino acid sequences of human, ape, dog, mouse, and rat PTKB2 are aligned. Numbers on left and right side indicate positions of amino acid residues. *K838.

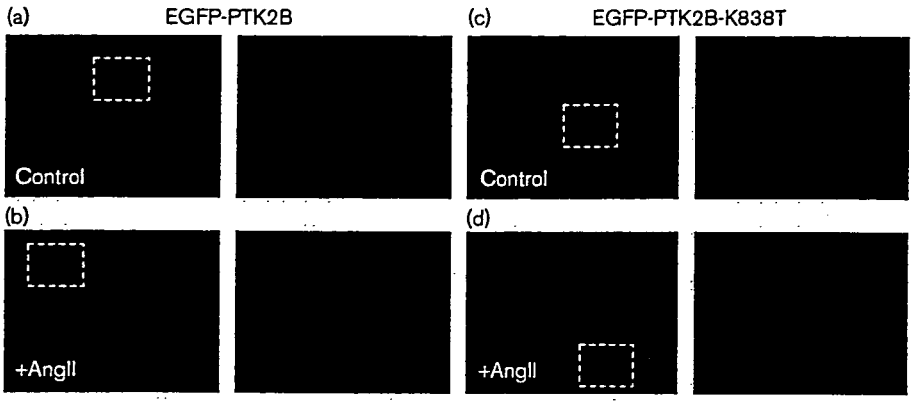
Discussion

In this study, we evaluated *PTK2B* as a candidate for a susceptibility gene for hypertension using population-based case–control samples including 3655 Japanese individuals (1520 patients with hypertension and 2135 controls). The multivariate logistic regression analysis after adjustments for confounding factors showed that −22A > G and 53484A > C (K838T) in *PTK2B* showed an association with the presence of hypertension in men. This association was stronger when hypertension was defined as SBP of ≥ 160 mmHg and/or DBP of ≥ 95 mmHg, or the current use of antihypertensive medication. Both SNPs were in LD with other polymorphisms in *PTK2B*, thus comprising an extensive haplotype block 113 kb in length. Therefore, this extensive haplotype block in *PTK2B* may be an important determinant for hypertension.

PTK2B is involved in the signaling pathways of Ang II and endothelin-1 (ET-1), two important vasoconstrictors, in cardiovascular cells [5,18,19], and nitric oxide, an important vasodilator, inhibited Ang II-induced activation of PTK2B [20]. In addition, PTK2B-mediated Ang II or ET-1-augmented migration and protein synthesis in VSMCs [17,21,22]. The augmented migration and protein synthesis by VSMCs could lead to medial thickening and progressive luminal narrowing of resistant blood vessels and result in hypertension [23,24]. Moreover, VSMCs from spontaneously hypertensive rats exhibited increased cell growth compared with those from normotensive Wistar–Kyoto rats [25], and increased PTK2B activity was involved in this effect [26]. All these results suggest that genetic variations of *PTK2B* influence the net-effects of vasoactive factors on VSMC phenotype and contribute to hypertension. Furthermore, *PTK2B* was originally identified in the human hippocampus and its mRNA was detected mainly in human brain and kidney [27]. An evidence to suggest that Ang II is a neurotransmitter and upregulation of the renin-angiotensin system in brain contributes to hypertension exists [28]. Therefore, an effect of genetic variations of *PTK2B* on the regulation of the cardiovascular system by the central nervous system is expected. Transgenic and knockout techniques for the *PTK2B* gene *in vivo* are necessary to clarify this point.

In this study, we genotyped six SNPs. Therefore, after applying the Bonferroni correction for multiple testing, the level of significance is $P < 0.0083$ (0.05/6 for 6 loci). −22A > G showed a significant association with hypertension in men ($P = 0.006$) even with use of a strict Bonferroni correction, when hypertension is defined as SBP of ≥ 160 mmHg and/or DBP of ≥ 95 mmHg, or the current use of antihypertensive medication. In addition, 53484A > C still showed a significant association with blood pressure levels in men ($P = 0.003$) after the Bonferroni correction. Power analysis also showed that these two SNPs, −22A > G and 53484A > C, had higher power more than 50%, and rest of SNPs did not have

Fig. 2



Fluorescent imaging of wild-type and mutant PTK2B molecules. HUVECs transfected with the plasmids encoding EGFP-PTK2B (a, b) and EGFP-PTK2B K838T (c, d) were starved for 4 h, and stimulated with vehicle (a, c) or 1 μmol/l Ang II for 5 min (b, d). Right side images of each panel show magnified view of the area in squares. HUVECs, human umbilical vascular endothelial cells.

power above 50%. Thus, *PTK2B* is speculated to be a susceptibility gene for hypertension.

The mechanisms by which the two SNPs (−22A > G and 53484A > C) might be associated with hypertension in men only are unknown. No association in women was observed. This inconsistency might be derived from sex differences. Regarding sex differences, the incidence and rate of progression of hypertension was markedly higher in men than in age-matched premenopausal women and, after menopause, this relationship no longer existed [29]. In various hypertensive animal models, males showed higher blood pressure levels than females owing to greater levels of Ang II-NADPH oxidase-mediated upregulation of the production of reactive oxygen species [30,31], Ang II-induced enhancement of sympathetic nerve activity [32], decreased nitric oxide production [33], and a high ratio of AT1/AT2 receptors of Ang II [34]. In addition, the elevation in blood pressure after the administration of ET-1 was much higher in male rats than in female rats [35], because estrogen might reduce ET-1-induced vasoconstriction [36]. As PTK2B is involved in the signaling pathways of Ang II and ET-1 and nitric oxide inhibits Ang II-induced activation of PTK2B [20], sex differences in the relationship between *PTK2B* polymorphisms and hypertension may be ascribed to the influences of these vasoactive factors.

The missense mutation K838T seemed to be important to the function of PTK2B. We expressed the mutant protein in mammalian cells and examined its intracellular localization by fluorescence imaging. It was clear that the mutant did not show extensive changes in terms of localization even after Ang II stimulation. This imaging, however, only looked at the localization. We have to examine the kinase activity of the mutant protein in a future study. In addition, −22A > G and SNPs in LD with 53484A > C (−99G > A, rs919495, rs11776858, rs11785606, rs10109834, and rs3735759) are present in the 5′-untranslational region. Whether they could influence *PTK2B* gene expression needs to be clarified.

In summary, we have found an association between hypertension and SNPs of *PTK2B*. Association-based studies are not consistently reproducible due to false positive results, false negative results, or true variability in associations among different populations [37]. Therefore, confirmation of the result in additional cohorts may be required.

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