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特集　高血圧治療における利尿薬の再評価

高齢者における利尿薬の評価*

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Key Words : elderly, hypertension, diuretics

はじめに

利尿薬は古い降圧薬で，その使用は減少傾向にあったが，より新しい降圧薬に優るとも劣らない大規模臨床試験の成績もあり，見直しの機運が高まっている．高齢者においても利尿薬の有用性が示されているが，副作用や使用上の注意点を認識しておく必要があろう．本稿では，高齢者高血圧治療における利尿薬について評価し，臨床試験からのエビデンスや，メリット，デメリットなどについて述べていきたい．

高齢者高血圧の特徴と降圧治療のポイント

加齢に伴い収縮期血圧は上昇し拡張期血圧は低下する傾向にあるため，高齢者では収縮期高血圧を呈する頻度が高い．また，高齢の高血圧症例は，脳血管障害，心不全，虚血性心疾患，腎硬化症など，多臓器にわたる高血圧合併症を有する頻度が高くなるだけでなく，加齢に伴う肝機能や腎機能の低下および神経反射機構による血圧調節機能の低下を有している．高齢者においても降圧薬による高血圧治療が心血管予後および生命予後を改善することは明らかであるが，薬物代謝・排泄が遅延しているため副作用が発現しやすいこと，血中濃度に依存して降圧効果を発揮する薬剤では過度の降圧をきたす可能性があることを肝に銘じておくべきである．すなわち，高齢者高血圧症例に対しては緩徐な降圧治療が重要で，そのためには降圧薬を少量から開始し，効果が不十分である場合には異なった作用機序の薬剤を併用し，降圧効果を高めて副作用発現をおさえるといった配慮が必要であると考えられる．

高齢者高血圧における降圧利尿薬のエビデンス

1．プラセボとの比較

高齢高血圧患者を対象として，利尿薬を基本とした降圧治療とプラセボを比較した検討が1970～1980年代に多施設大規模臨床研究として複数行われ，予後改善を示す結果が報告されている．

EWPHE(European Working Party on High Blood Pressure in the Elderly)は，60歳以上，平均75歳の高血圧症例840名を対象に，平均4.6年にわたり，総死亡，心血管疾患死亡，心臓死の頻度について，利尿薬(ヒドロクロロチアジド，トリアムテレン)による治療群とプラセボ群との比較を行った大規模臨床試験である．総死亡に有意差は認められなかったが，心血管疾患死亡は利尿薬群で27%少なかった[1]．

SHEP(Systolic Hypertension in the Elderly

* Diuretics in hypertensive elderly patients.

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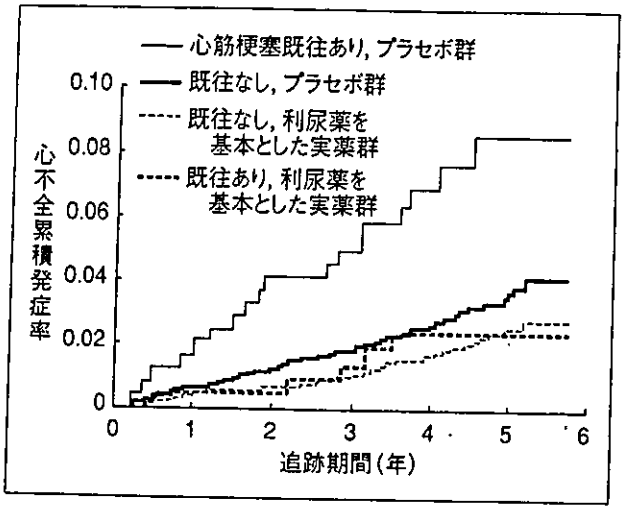

図1 SHEP研究における心不全累積発症率の経過（文献[2]より引用）

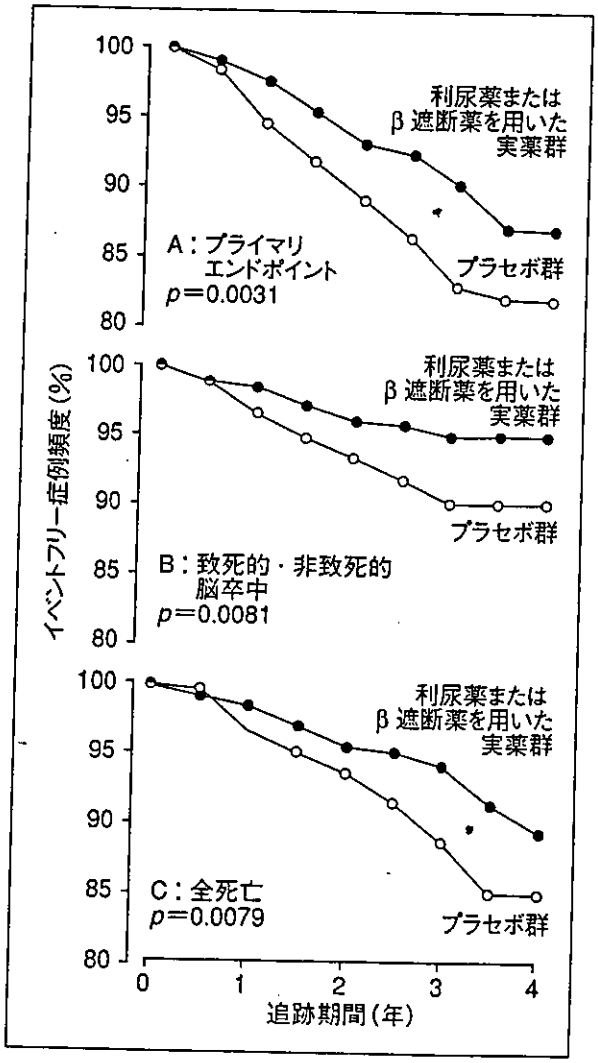

図2 STOP-Hypertension研究におけるイベントフリー症例頻度の経過（文献[4]より引用）

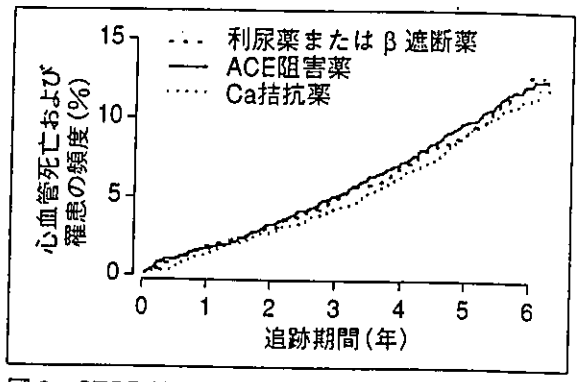

図3 STOP-Hypertension 2研究における心血管疾患死亡率および罹患率の経過（文献[6]より引用）

Program）は，60歳以上，平均72歳の収縮期高血圧の症例4,736名を対象として，平均4.5年にわたり，利尿薬クロルタリドン（＋β遮断薬アテノールまたはレセルピン）による治療群とプラセボ群との比較を行った臨床試験である．利尿薬を基本とした降圧治療は，心不全の発症および心不全死を49%減少させた．とくに，心筋梗塞の既往を有する症例群では心不全リスクが高いが，治療群では実に81%のリスク減少を認めている（図1）[2]．さらに，治療群では全脳卒中発症を36%減少させただけでなく，冠血管死と非致死的心筋梗塞の合計を27%減少させた[3]．本研究は，降圧薬治療が脳卒中だけでなく，虚血性心疾患の抑制においても臨床上有用であることを示している．

STOP-Hypertension（Swedish Trial in Old Patients with Hypertension）では，70～84歳の高齢者高血圧症例1,627名を対象として，平均25か月にわたり，利尿薬（ヒドロクロロチアジドおよびアミロライドの併用）またはβ遮断薬を用いる実薬群とプラセボ群との比較を行った臨床試験である．実薬群ではプラセボ群と比較して，脳卒中，心筋梗塞，その他の心血管死の総頻度（プライマリエンドポイント）は40%少なかった．各疾患別では，脳卒中が47%，心筋梗塞が23%，その他の心血管死が70%といずれも有意に減少していた[4]．また，全死亡数も43%の有意な減少を認めた（図2）．よって，より高齢の高血圧症例においても降圧療法を行う意義があることが示唆された．

MRC（Medical Research Council）trailでも，高齢者において利尿薬（ヒドロクロロチアジドとア

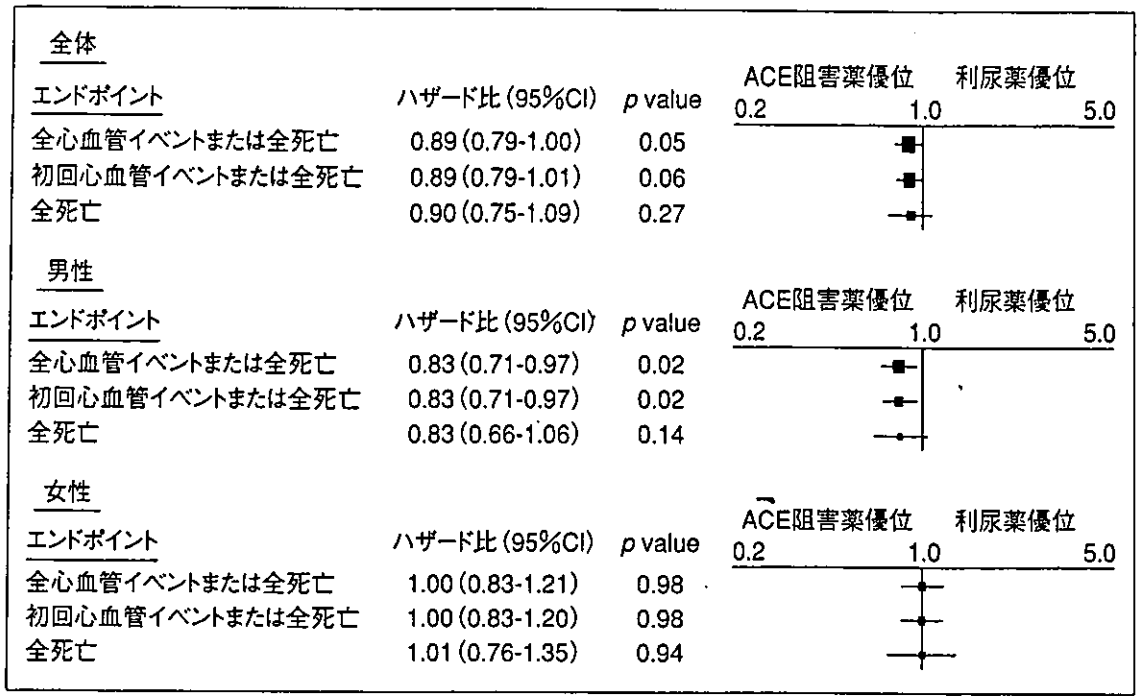

図4 ANBP2研究における心血管イベントおよび全死亡のハザード比(文献[7]より引用)

ミロライドの併用)またはβ遮断薬(アテノロール)を用いる実薬群はプラセボ群と比較して，脳卒中の発症が25%少なかったことが報告されている[5]．

2．ほかの降圧剤との比較

STOP-Hypertension 2は，高齢者高血圧症例に対して利尿薬またはβ遮断薬と，Ca拮抗薬およびACE阻害薬の心血管系死亡率および罹患率への影響を比較した臨床研究である．年齢70～84歳，平均76歳の高齢者高血圧症例6,614名を対象に，4～6年間の追跡期間にわたり施行された．心血管死亡率，致死的・非致死的心血管疾患の発症率において3群間に有意差を認めなかった(図3)[6]．すなわち，利尿薬とβ遮断薬は，Ca拮抗薬やACE阻害薬と同等に高齢者高血圧症例の心血管系死亡率および罹患率を低下させることが示されたことになる．

ANBP2(Second Australian National Blood Pressure Study)は，年齢65～84歳，平均72歳の高血圧症例6,083名を対象として，約4年にわたり，心血管系イベントおよび全死亡について，利尿薬ヒドロクロロチアジドを主とした降圧療法群とACE阻害薬エナラプリルを主とした群との比較を行った臨床研究である．降圧効果は両群で同等であり，全心血管系疾患の発生率および全死亡の合計は利尿薬群が多い傾向を示し，男性では有意であった(図4)[7]．この研究は，利尿薬がACE阻害薬よりやや劣ることを示唆しているが，女性で差はなく，さらなる検討が必要であると考えられる．

3．その他の大規模臨床試験

高齢者のみを対象としない高血圧治療の大規模臨床試験のいくつかは高齢者を含んでおり，それらにおいても利尿薬による予後改善が示されている．また，脳卒中の再発予防に関しても利尿薬の有用性が明らかとなってきた．

別項で詳述されるALLHAT(Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial)は，年齢55歳以上の高血圧症例4万人以上を対象として，利尿薬クロルタリドンとCa拮抗薬アムロジピン，ACE阻害薬リシノプリルおよびα遮断薬ドキサゾシンを比較した臨床研究である．ドキサゾシン群は心不全および全心血管イベントがクロルタリドン群より有意に多く，早期に中止された．クロルタリドン群は，アムロジピン群やリシノプリル群と比較して，冠動脈疾患死および非致死的心筋梗塞の発生率に差はなく，全心血管イベントの発生率はリシノプリル群より有意に少なく，心不全の発生率はアムロジピン群，シリノプリル群と比べて有

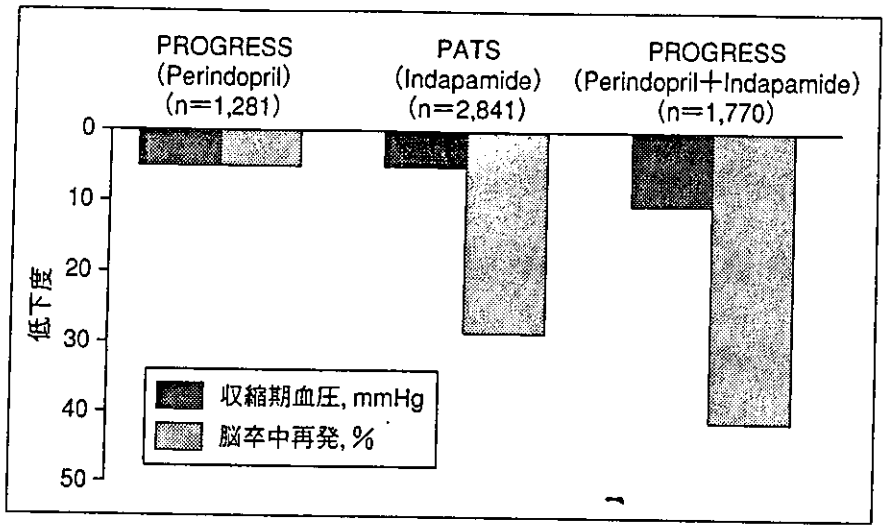

図5 PROGRESS, PATS研究における脳卒中抑制効果(文献[11]より引用)

意に低値であった[8]. しかしながら, 代謝性副作用に関しては, クロルタリドン群がほかの2群に比べ, 治療後のコレステロール値, 空腹時血糖値は有意に高く, 血清カリウム濃度は低かった. 糖尿病を新規発症したものもクロルタリドン群が多かった. よって, ALLHATは必ずしも利尿薬の臨床上の有用性を示すだけでなく, 臨床上の問題点も含めて浮き彫りにした報告であると考えられる.

PATS(Post-stroke Antihypertensive Treatment Study)は, 中国で行われた脳卒中の再発予防についての利尿薬を用いた大規模臨床試験である. 対象は高齢者に限らないが, 2,841名の脳卒中患者で利尿薬インダパミドあるいはプラセボが投与され, 3年間追跡された. インダパミド群はプラセボ群より収縮期血圧は5 mmHg低下し, 脳卒中再発は29%減少しており, 利尿薬による降圧治療が脳卒中患者の予後を改善することが示された(図5)[9].

PROGRESS(Perindopril Protection Against Recurrent Stroke Study)は, ACE阻害薬を基本薬とした脳卒中の再発予防についての国際的大規模研究である[10]. この研究も対象は高齢者や高血圧症例に限定されてはいないが, 平均年齢64歳の脳卒中患者6,105名がペリンドプリルまたはプラセボに無作為に割り付けられ, ペリンドプリル群で血圧コントロールが不十分の場合には利尿薬インダパミドが追加された. 4年間の脳卒中再発は実薬により28%減少したが, ペリンドプリル単独群では, 収縮期血圧の低下は5 mmHgで脳卒中再発への効果は−5%にすぎず, 有意ではなかった. 一方, インダパミドの併用群では収縮期血圧の低下も大きく(−12mmHg), 脳卒中再発は43%減少している(図5). したがって, 脳血管障害を伴う高齢高血圧患者において, 利尿薬による治療はきわめて有用であると考えられる[11].

高齢者高血圧における利尿薬の位置づけ

最近の高血圧治療のガイドラインにおいて, 利尿薬は高齢者における使用薬剤として推奨されている.

わが国では2000年の高血圧治療ガイドライン[12], および2002年の老年者高血圧治療ガイドライン[13]のいずれにおいても, 少量の利尿薬を, Ca拮抗薬, ACE阻害薬, アンジオテンシンII受容体拮抗薬とともに第一選択薬として勧めている(図6). また, 合併症を有する高齢高血圧患者においては, 利尿薬は脳血管障害, 心不全, 腎障害, 骨粗鬆症の場合によい適応, 糖尿病, 高脂血症, 閉塞性動脈硬化症では要注意, 通風があれば禁忌としている.

米国のJNC-7(2003年)は, 高血圧の薬物療法において積極的適応がない場合には利尿薬を第一選択薬として推奨しており, 併用療法においても利尿薬を含めることを勧めている[14]. 利尿薬はまたいくつかの合併症についての積極的適応に

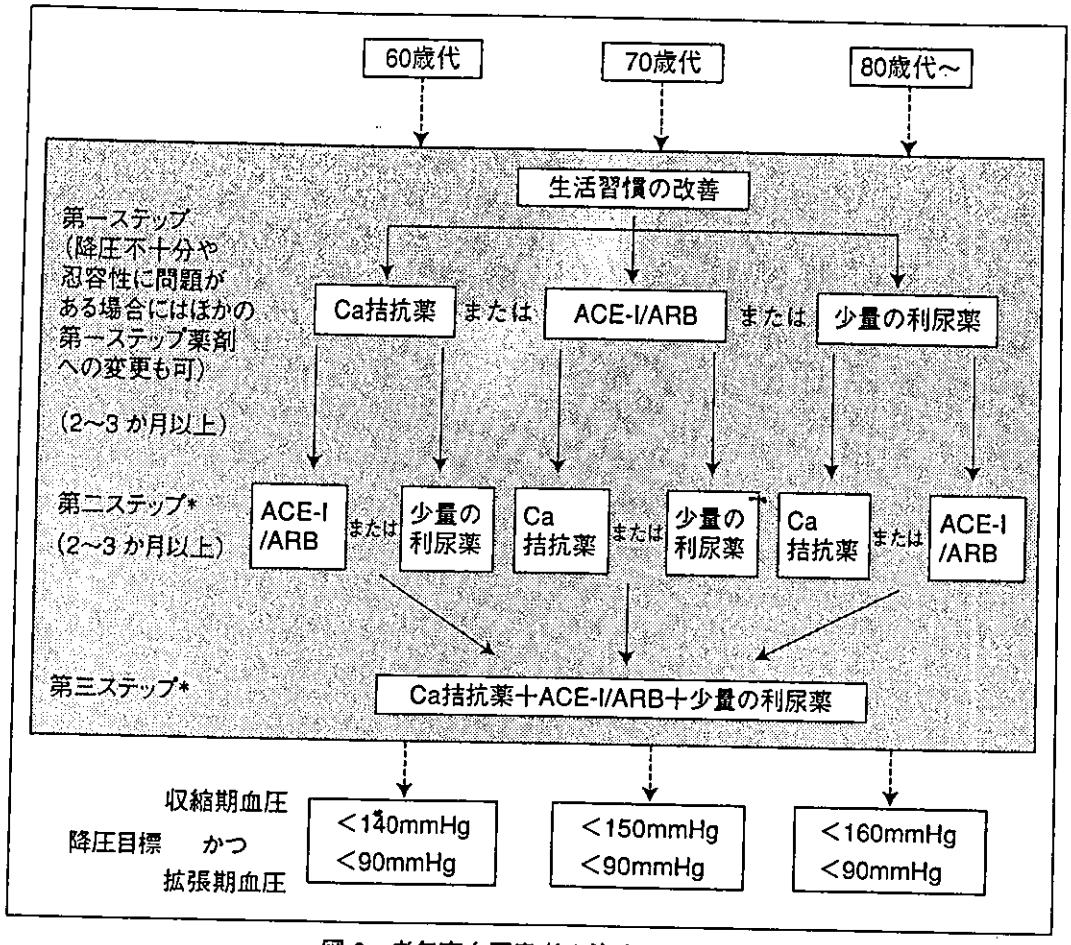

図6　老年高血圧患者の治療チャート
ACE-I：アンジオテンシンⅠ変換酵素阻害薬，ARB：アンジオテンシンⅡ受容体拮抗薬，ACE-I/ARB：ACE-IまたはARB．＊症例によりβ遮断薬も使用可能である．　（文献[13]より引用）

あげられる．高齢者においても，これらの原則は同様であるとしている．

ヨーロッパのESH/ESCのガイドライン(2003年)も，高齢高血圧患者の治療は概括的なガイドラインに従うべきと述べており，利尿薬はほかの主要な降圧薬とともに降圧薬治療の開始および維持に適していることを支持している[15]．

高齢者における利尿薬の副作用と注意点

利尿薬の投与により低カリウム血症，低ナトリウム血症，低クロール性アルカローシス，低マグネシウム血症などをきたす可能性がある．低カリウム血症は低マグネシウム血症とともに，心筋の被刺激性亢進や不整脈の誘発を介して突然死への誘因のひとつとなる危険性を有する．SHEPのサブ解析では，低カリウム血症群は血清カリウム正常群と比較して，降圧は同程度でも，クロルタリドンによる心血管系リスクの改善効果が減弱することが示された[16]．したがって，利尿薬を用いる場合には低カリウム血症への注意を要し，カリウム製剤やカリウム保持性利尿薬の併用などを考慮すべきであろう．

利尿薬は腎尿細管における尿酸分泌を抑制するため，高尿酸血症をきたす．SHEPのサブ解析では，尿酸値の上昇が0.06mM以上だった群では，0.06mM未満の群と比較して，利尿薬クロルタリドンによる冠イベントリスクの改善効果が減弱することが報告された[17]．高尿酸血症を伴う患者においては，利尿薬はよい適応ではなく，また，利尿薬による尿酸値上昇が著しい場合には，減量や中止あるいは尿酸低下薬の併用などの対策を要する．

利尿薬は少量でもCa拮抗薬やACE阻害薬と比較して耐糖能異常を促進しやすいことが，ALLHATなどにおいて明らかとなった．しかしながら，

SHEPのサブ解析においては，非糖尿病症例だけでなく，糖尿病を合併した症例に対しても，利尿薬が心血管系イベント抑制効果を有することが報告された[18]．よって，利尿薬は耐糖能悪化に留意すべきであるが，糖尿病を伴う例においても禁忌ではないと考えられる．

降圧利尿薬の降圧および利尿作用に対する用量－反応曲線は平坦であるのに対して，代謝系の副作用に関しては用量依存性である．よって，利尿薬の用量は可能なかぎり少量にとどめ，十分な降圧が得られない場合には，増量するよりも降圧機序の異なるほかの降圧薬を併用する方が望ましい．

利尿薬の代謝への作用は必ずしも悪いことばかりではない．サイアザイド系の薬剤はカルシウムの尿中排泄を減らし，血中カルシウム値を上げ，骨量を増加させ，骨折予防に働くことが示されている[19][20]．骨粗鬆症をきたしやすい高齢女性にとって，利尿薬は寝たきりの主要な原因となる脳卒中と骨折をともに防ぐ有用な薬剤といえよう．

おわりに

高齢者における利尿薬の評価について述べた．利尿薬による治療が，高齢高血圧患者の脳卒中や心筋梗塞，心不全などを予防し，生命予後を改善することは明らかである．高齢化や医療費増加の折，安価で，少量であれば副作用は少なく，ほかの多くの降圧薬と相加的，相乗的にはたらく利尿薬は，使用が見直されるべきであろう．利尿薬は代謝面の副作用に注意を要するが，一方ではカルシウム代謝を介して高齢者の骨折予防にはたらくという好影響にも留意しておく必要があろう．

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Original Article

The Thiazide-Sensitive Na^+-Cl^- Cotransporter Gene, *C1784T*, and Adrenergic Receptor-β3 Gene, *T727C*, May Be Gene Polymorphisms Susceptible to the Antihypertensive Effect of Thiazide Diuretics

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The response of blood pressure to thiazide diuretics (TZDs) differs among individuals. The prediction of the antihypertensive effect of TZDs is important for realizing individualized therapy in the management of hypertension. The aim of this study was to identify the single nucleotide polymorphisms (SNPs) susceptible to the antihypertensive effect of TZDs, particularly focusing on genes related to water-electrolyte absorption in the kidney. Seventy-six outpatients (mean age, 65.4±9.0 years) with essential hypertension (EHT) taking TZDs were retrospectively assessed. We defined as responders (R) those whose mean blood pressure was lowered by more than 5 mmHg after the use of TZDs. Forty-eight SNPs in 17 genes (*ADD1*, *GNB3*, *TSC* [*SLC12A3*], *MLR* [*NR3C2*], *NCX1* [*SLC8A1*], *WNK1*, *WNK4*, *AGT*, *ACE*, *AT1* [*AGTR1*], *CYP11B2*, *ADRB1*, *ADRB2*, *ADRB3*, *ADRA1A*, *ADRA1B*, *ADRA2A*) were genotyped in the 76 patients. The SNPs in *TSC*, *MLR*, *NCX1*, *WNK1*, and *WNK4* were identified by direct sequencing and those with minor frequencies of greater than 5% were genotyped in this study. The comparison of polymorphism prevalence between R and non-responders (NR) showed significant differences in *TSC C1784T* (*C* allele *vs.* *T* allele, odds ratio (OR)=3.81, p=0.016, confidence interval (CI): 1.25–11.63) and *ADRB3 T727C* (*Trp64Arg*) (*T* allele *vs.* *C* allele, OR=4.59, p=0.005, CI: 1.54–13.68). The blood pressure (BP) in patients homozygous for the major alleles of both *TSC C1784T* and *ADRB3 T727C* were significantly reduced by TZD treatment; however, the BP in those homozygous for the minor allele and heterozygous (*TSC C1784T*: TT+CT; *ADRB3 T727C*: CC+CT) for both SNPs were not significantly changed after TZD treatment. Both newly detected *TSC C1784T* and *ADRB3 T727C* are gene polymorphisms susceptible to the antihypertensive effect of TZDs in patients with EHT. Thus, the prediction of BP reduction by TZDs may be possible by evaluating these two SNPs.

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***Key Words*: thiazide diuretics, gene polymorphism, essential hypertension**

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Introduction

Thiazide diuretics (TZDs) have been most widely used as a first line antihypertensive drug (*1*, *2*). Recently, the ALLHAT study confirmed the usefulness of TZDs for the reduction of blood pressure (BP) and cardiovascular diseases in comparison with newer antihypertensive drugs, including Ca channel blockers (CCBs) such as amlodipine and angiotensin converting enzyme inhibitors (ACEIs) such as lisinopril in about 40,000 hypertensive patients with high risk factors (*3*). TZDs are not only effective as a monotherapy for hypertension, but are also very useful for combination therapy with other antihypertensive drugs (*4*). Moreover, the use of a TZD as a drug therapy for hypertension, which is a chronic and lifelong disease, would be very good from the viewpoint of the cost of drugs, because TZDs are the cheapest of all antihypertensive drugs. However, the response of BP to TZDs differs among individuals, and TZDs often induce side effects, such as hypokalemia and lipid, glucose and uric acid metabolism abnormalities (*4*). Therefore, it would be useful to determine the individual sensitivity to a TZD before prescribing it.

Regarding previous findings about gene polymorphisms that influence TZD-sensitivity, Turner *et al.* (*5*) reported that the β3-subunit of the G protein (*GNB3*) *C825T* polymorphism was related to the antihypertensive effect of a TZD in Caucasian and African-American subjects with essential hypertension (EHT). Glorioso *et al.* (*6*) also demonstrated that the α-adducin (*ADD1*) *Gly460Trp* polymorphism is the gene conferring susceptibility to the antihypertensive effect of TZDs in Italian hypertensives. This *ADD1 Gly460Trp* polymorphism was also suggested to confer susceptibility to salt-sensitivity in Caucasians and Asians with EHT (*7*).

Mutations of causative genes have recently been detected in several monogenic electrolyte disorders, such as mutations in the thiazide-sensitive Na-Cl cotransporter (*TSC*) gene for Gitelman syndrome (*8*, *9*), the WNK1 and 4 genes for Gordon syndrome (pseudohypoaldosteronism type II) (*10*) and the mineral corticoid receptor (*MLR*) for pseudohypoaldosteronism type I (PHA I) (*11*). TZDs are commonly effective for treating Gitelman syndrome and Gordon syndrome. We also focused on the Na^+/Ca^{2+} exchanger gene (*NCX1*), because its impairment was recently reported in mesangial cells from salt-sensitive hypertensive rats (*12*). TZDs are known to be effective for salt-sensitive hypertension. It is also known that the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) are activated in response to changes in circulating blood volume after TZD administration. Therefore, it is expected that gene polymorphisms related to the RAAS and SNS might be involved in the antihypertensive effect of TZDs. The present study investigated the gene polymorphism influencing the TZD-sensitivity by analyzing mainly single nucleotide polymorphisms (SNPs) of several water-electrolyte-related genes, including *GN3B*, *ADD1*, *TSC*, *MLR*, *NCX1*, *WNK1*, *WNK4* and RAAS- and SNS-related genes, to anticipate the effect of TZDs on BP in patients with hypertension.

Methods

Study Subjects

Peripheral blood samples for genetic analysis were collected with written informed consent from Japanese patients with EHT at an outpatient clinic of the Division of Hypertension and Nephrology, National Cardiovascular Center, Suita, Japan. This study protocol was approved by the Ethical Committee of the National Cardiovascular Center. Seventy-six patients, who had been newly prescribed TZDs as monotherapy or in addition to other antihypertensive agents, and whose blood-pressure data could be obtained from patients' records in 3 consecutive outpatient visits before and after starting TZDs, were retrospectively enrolled. BP was measured in the subjects after at least 10 min of rest in a sitting position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were the means of three physician-obtained measurements. All subjects visited the outpatient clinic every month. The TZDs were a combination of indapamide (64.5%), trichlormethiazide (26.3%), mefruside (7.9%), and hydrochlorothiazide (1.3%). We defined patients who could achieve a BP reduction greater than 5 mmHg of mean blood pressure (MBP) after taking the TZDs as responders (R), and patients who could not achieve a BP reduction greater than 5 mmHg of MBP or showed increased BP after taking TZDs as non-responders (NR), according to the common evaluation criteria of antihypertensive drug effectiveness in Japan.

DNA Studies

Direct Sequencing for Detection of Polymorphisms in TSC, MLR, WNK1, WNK4, *and* NCX1

Genomic DNA was extracted using an NA-3000 nucleic acid isolation system (KURABO, Osaka, Japan) and stored at −80 °C until use. We sequenced the 32–48 Japanese samples with written informed consent for genetic analysis. The methods used for the direct sequencing have been described previously (*13*). Briefly, all exons, part of the intron and an approximately 1,000-bp upstream region of exon 1, which would include the promoter regions of the *TSC*, *WNK1*, *WNK4* and *NCX* genes, were individually amplified by polymerase chain reaction and sequenced on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA). In the *MLR* gene, exon 2 was sequenced. The polymorphisms were identified using the Sequencer software package (Gene Codes Corp., Ann Arbor, USA), followed by visual inspection.

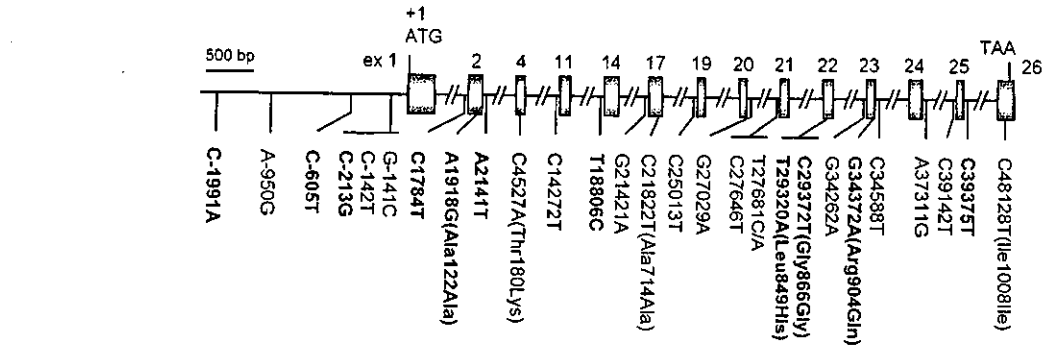

Fig. 1. *Location of genetic variants identified in* TSC. *Nucleotide numbers were counted from the initiation codon (ATG). Sequencing regions are indicated by the bars above the schematic gene structure. The initiation codon, ATG, and stop codon, TAA, are also shown. The single nucleotide polymorphisms presented in bold were genotyped in this study.*

Genotyping of Polymorphisms

The polymorphisms were genotyped using the TaqMan-polymelase chain reaction (PCR) system. Regarding genotyped SNPs, *C825T* of *GNB3* and *G29071T-Gly460Trp* of *ADD1* were selected according to previous studies (*5*, *6*, *14*). As the RAAS-related genes, *angiotensinogen* (*AGT*: *A−20C*, *G−6A*) (*15*, *16*), *ACE* (*G12568C* for *I/D*) (*17*), *angiotensin II type 1 receptor* (*AT1*: *A1166C*, *A−153G*) (*18*, *19*), and *aldosterone synthase* (*CYP11B2*: *C−344T*) (*20*) were tested. Furthermore, as the SNS-related genes, *adrenergic receptor β-1* (*ADRB1*: *A393G-Ser49Gly*, *G1413C-Arg389Gly*) (*21*), *β-2* (*ADRB2*: *C−47T*, *G2118A-Gly16Arg*, *G2151C-Glu27Gln*) (*22*, *23*), *β-3* (*ADRB3*:*T727C-Trp64Arg*) (*24*), *α-1a* (*ADRA1A*: *T44653C-Arg492Cys*) (*25*), *α-1b* (*ADRA1B*: *G834A*, *G1167A*) (*26*) and *α-2a* (*ADRA2A*: *A3023G*) (*27*) were tested for TZD sensitivity. Regarding *ACE*, we genotyped *G12568C* instead for the *I/D* polymorphism of 287 bp in intron 16 because *G12568C* showed almost complete linkage disequilibrium (LD) with the *I/D* polymorphism, as reported previously (*17*). For the directly sequenced genes, SNPs having a minor allele frequency of greater than 5% were selected for genotyping. As a result, 11 SNPs of *TSC* (Fig. 1), 2 SNPs of *MLR*, 7 SNPs of *WNK1*, 2 SNPs of *WNK4* and 7 SNPs of *NCX1* were genotyped. The sequences of the allele-specific probes and PCR primers used for the genotyping are shown in Table A1 in Appendix.

Statistical Analysis

Values are expressed as the means±SD. Hardy-Weinberg equilibrium was assessed by χ^2 analysis. The overall distribution of alleles was analyzed by χ^2 analysis. The distribution of genotypes between R and NR was analyzed by 2×2 contingency tables with a 2-sided Fisher exact probability test. The statistical significance was established at $p<0.05$. Comparison of BP reduction between allelic variants was performed by ANOVA followed by the Fisher protected least significant difference test using Stat-View version 5.0 (SAS Institute Inc., Cary, USA). LD and haplotype analyses were performed using the SNPAlyze statistical package version 2.1 (DYNACOM Co., Ltd., Mobara, Japan). The LD between SNPs was calculated by r^2. Tight LD was regarded as $r^2 \geq 0.5$. Haplotype estimation was performed by the expectation-maximization algorithm.

Results

Group Characteristics

Overall BP was significantly reduced after TZD administration (Fig. 2). Table 1 shows the group characteristics of R and NR. Forty-five patients who showed an MBP reduction of greater than 5 mmHg were defined as R, and 31 patients were defined as NR. Neither averaged age nor body mass index (BMI) showed a significant difference between R and NR. The BP before TZD administration was significantly higher in R than in NR. After TZD treatment, the averaged BP in R was remarkably decreased; however, the averaged BP in NR was slightly higher than that at pretreatment (Table 1). Control for deviation from Hardy-Weinberg equilibrium gave non-significant results in most SNPs examined in the present study, except *ADRA1B G1167A*. In the genotyping of *ADRA1B G1167A*, all of the study subjects were homozygous for the major allele, GG. This suggests that polymorphism of *ADRA1B G1167A* might not exist in the Japanese population.

Detection of Genetic Variants

We detected 52 SNPs of *TSC*, 7 SNPs of *MLR*, 35 SNPs of *WNK1*, 22 SNPs of *WNK4* and 15 SNPs of *NCX1* (Table A2 in Appendix). We confirmed some of the identified SNPs in the public database, dbSNPs (http://www.ncbi.nlm.nih.gov/SNP/). As shown in Table A2 in Appendix, some SNPs were very rare. Therefore, we chose SNPs that had a minor allele frequency of greater than 5% for genotyping by the TaqMan

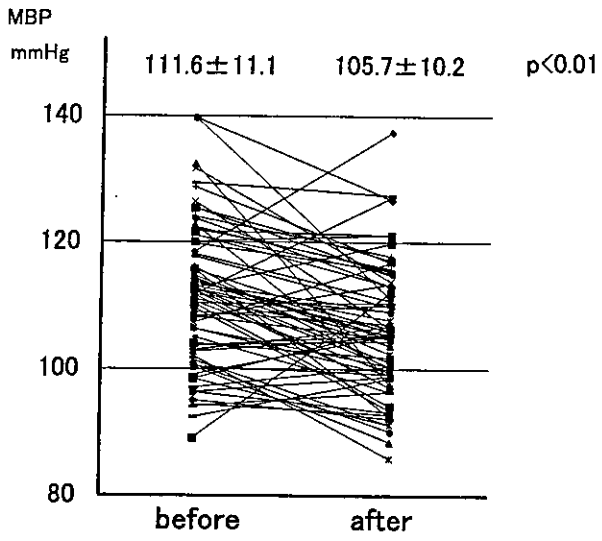

Fig. 2. *Mean blood pressure (MBP) before and after treatment with thiazide diuretics (TZDs) in all subjects. The average MBP in all subjects was significantly reduced by treatment with TZDs.*

method. Furthermore, some of these polymorphisms showed LD with other polymorphisms. Thus, we chose one SNP from among the polymorphisms with tight LD (*28*) ($r^2 \geq 0.5$) for genotyping the subjects. Therefore, genotyping by the TaqMan method was finally performed for 11 SNPs in *TSC*, 2 SNPs in *MLR*, 7 SNPs in *WNK1*, 2 SNPs in *WNK4* and 7 SNPs in *NCX1* (Table A1 in Appendix).

Association Study for the Effect of TZDs

No polymorphisms of the *MLR*, *WNK1*, *WNK4*, *NCX1*, and RAAS genes, and no polymorphisms of most of the SNS genes examined in the present study, including *C825T GNB3* and *Gly460Trp ADD1*, were significantly related to the effect of the TZDs, based on the comparison of each allele frequency between R and NR (Table 2). Two SNPs, *TSC C1784T* and *ADRB3 T727C*, showed a significant correlation with the effect of the TZDs (Table 3). The BP in patients with the homozygotes of the major allele of both *TSC C1784T* and *ADRB3 T727C* were significantly reduced by TZD treatment; however, the BP in those with the homozygotes of the minor allele and heterozygote (*TSC C1784T*: TT+CT; *ADRB3 T727C*: CC+CT) of both SNPs were not significantly changed after TZD treatment (Fig. 3). Furthermore, there was a much more significant difference in prevalence between the patients with the homozygotes of the minor alleles and heterozygote of these two SNPs, *TSC C1784T* and *ADRB3 T727C*, combined and those with the homozygotes of the major allele in R and NR than in *TSC C1784T* or *ADRB3 T727C* alone (odds ratio [OR]=5.09, $p=0.003$, confidence interval [CI]: 1.82–14.23).

Table 1. Comparison of Patients Characteristics between R and NR of TZD

	R (±SD)	NR (±SD)	*p* value
Number	45	31	
Age (years)	64.5±9.3	66.7±8.6	N.S.
Sex (male/female)	18/27	20/11	<0.05
BMI (kg/m^2)	24.7±3.2	24.7±3.2	N.S.
Pre SBP (mmHg)	157.9±14.3	142.9±15.0	<0.01
Pre DBP (mmHg)	95.0±10.7	86.5±10.1	<0.01
Pre MBP (mmHg)	115.9±9.7	105.3±9.9	<0.01
Pre HR (/min)	71.0±7.9	72.4±7.9	N.S.
Post SBP (mmHg)	138.8±15.0	147.2±17.4	<0.05
Post DBP (mmHg)	87.0±9.9	88.2±11.0	N.S.
Post MBP (mmHg)	104.3±9.3	107.9±11.2	N.S.
Post HR (/min)	72.4±9.3	72.8±9.0	N.S.
Monotherapy (%)	28.9	25.8	N.S.
Kind of TZD (%)			
Indapamide	66.7	61.3	N.S.
Trichlormethiazide	26.7	25.8	N.S.
Mefruside	6.7	9.7	N.S.
Hydrochlorothiazide	0.0	3.2	N.S.

R, responder; NR, non-responder; TZD, thiazide diuretics; BMI, body mass index; Pre SBP, systolic blood pressure at pretreatment; Pre DBP, diastolic blood pressure at pretreatment; Pre MBP, mean blood pressure at pretreatment; Pre HR, heart rate at pretreatment; Post SBP, systolic blood pressure at posttreatment; Post DBP, diastolic blood pressure at posttreatment; Post MBP, mean blood pressure at posttreatment; Post HR, heart rate at posttreatment; Monotherapy, prevalence of monotherapy by thiazide diuretics; Kind of TZD, prescribed kinds of TZD; N.S., not significant.

Haplotype Analysis

We measured the LD to understand the haplotype distribution of *TSC C1784T* in Japanese. There was a strong LD between the multiple SNPs within *TSC*. *TSC* was composed of three LD blocks, and the LD block containing *C1784T* consisted of two SNPs, *C−213G* and *C1784T*. The haplotype frequency was calculated for these two SNPs and the differences in haplotype distribution were compared between R and NR (Table 4). The results showed that the haplotype H2 with the 1784T allele tended to be different between the two groups, although not significantly so ($p=0.094$).

Discussion

The present study demonstrated that *TSC C1784T* and *ADRB3 T727C* were associated with the antihypertensive effect of TZDs in Japanese patients with EHT. A hypertensive patient with the minor homozygote or heterozygote of these two SNPs is predicted to be a non-responder to TZDs.

The TSC is present in the distal convoluted tubule, which

Table 2. Comparison of Allele Frequency between R and NR to TZD

Gene	Minor allele *vs.* common allele			
	SNP	Odds ratio	*p*	95% CI
ADDI	*Gly460Trp*	1.300	0.427	0.680–2.487
GNB3	*C825T*	1.620	0.146	0.844–3.110
TSC	*C−1991A*	1.483	0.586	0.356–6.167
	C−605T	1.483	0.586	0.356–6.167
	C−213G	1.483	0.586	0.356–6.167
	C1784T	3.816	0.013	1.253–11.627
	A1918G	0.594	0.235	0.251–1.410
	A2141T	2.378	0.102	0.821–6.886
	T18806C	0.825	0.566	0.428–1.591
	T29320A	1.459	0.790	0.090–23.770
	C29372T	1.143	0.718	0.554–2.359
	G34372A	—	0.147	—
	C39375T	0.681	0.311	0.323–1.436
MLR	*C−2G*	1.147	0.731	0.524–2.509
	G538A	0.685	0.418	0.274–1.716
WNK1	*G786A*	1.021	0.965	0.407–2.561
	C108560T	1.026	0.967	0.310–3.400
	A128177C	1.124	0.791	0.473–2.673
	C133634T	1.189	0.721	0.461–3.067
	G135642T	0.950	0.881	0.484–1.864
	C141114T	0.820	0.617	0.377–1.785
	C142763T	0.967	0.971	0.157–5.961
WNK4	*C14597T*	1.467	0.704	0.201–10.700
	C14717T	1.780	0.287	0.609–5.203
NCX1	*T−23690C*	0.849	0.721	0.346–2.084
	C−23449A	0.864	0.846	0.199–3.757
	T−23200C	0.651	0.553	0.156–2.711
	T−23181C	0.850	0.633	0.436–1.656
	A−22729C	0.914	0.861	0.334–2.505
	C−22144G	2.967	0.357	0.263–33.454
	G252581A	0.906	0.779	0.456–1.802
AGT	*A−20C*	1.265	0.540	0.596–2.687
	G−6A	0.758	0.527	0.320–1.793
ACE	*G12568C (I/D)*	0.768	0.443	0.392–1.508
AT1-R	*A1166C*	0.712	0.639	0.171–2.961
	A−153G	1.172	0.818	0.302–4.552
CYP11B2	*C−344T*	1.554	0.219	0.768–3.145
ADRB1	*G1413C*	1.724	0.228	0.707–4.204
	A393G	0.692	0.432	0.276–1.738
ADRB2	*C−47T*	1.098	0.869	0.361–3.338
	G2118A	1.531	0.203	0.793–2.956
	G2151C	1.228	0.744	0.358–4.217
ADRB3	*T727C*	4.591	0.003	1.541–13.680
ADRA1A	*T44653C*	0.630	0.412	0.207–1.913
ADRA1B	*G834A*	1.381	0.333	0.718–2.657
	G1167A	—	—	—
ADRA2A	*A3023G*	1.223	0.556	0.626–2.389

SNP, single nucleotide polymorphism; R, responder; NR, non-responder; TZD, thiazide diuretics; CI, confidence interval.

has been suggested to be the principal mediator of sodium and chloride reabsorption in this segment of the nephron. Simon *et al.* (*9*) demonstrated complete linkage of Gitleman syndrome to the genetic variants of *TSC*. The loss of function of TSC in patients with *TSC* gene variants could lead to low blood pressure, hypokalemic alkalosis, hypomagnesaemia and hypocalciuria (*29*). Melander *et al.* reported that gene polymorphism of TSC may influence EHT (*30*). It has recently been reported that TSC might interact with WNK4, which is one of the causative genes of Gordon syndrome (*31*, *32*). TZDs are generally effective in patients with Gordon syndrome. This would be due to the interaction between WNK and TSC. Four kinds of TZDs were used for the present study, and the main pharmacological mechanism of the antihypertensive effect common among those four TZDs was the blockage of TSC in the distal tubule. For these reasons, we expected that the gene polymorphisms of *TSC*, *WNK1* and *WNK4* might be related to the effect of TZDs. One SNP of *TSC* showed a significant association with the effect of the TZDs; however, there were no positive SNPs in either *WNK1* or *WNK4*.

TSC consists of 26 exons and is located on 16q13. We performed direct sequencing for *TSC*, including the promoter region, and detected 52 SNPs. We screened 11 SNPs that showed a minor allele frequency of greater than 5% for TZD sensitivity (Fig. 1). As a consequence, only *TSC C1784T* showed a significant correlation with the effect of TZDs. Since *TSC C1784T* is located in intron 1, as shown in Fig. 1, this SNP itself may not influence TSC function. Although we could not find functional polymorphisms linked with *C1784T*, there may be functional polymorphisms in much further upstream regions of the promoter or genes adjacent to *TSC*.

Administration of a TZD often induces activation of the RAAS and/or SNS as a result of circulating volume reduction. Thus, we investigated the participation of gene polymorphisms of the RAAS and SNS. The gene polymorphisms were selected from previous studies investigating the correlation between BP regulation and gene polymorphisms (*33*). Although Sciarrone *et al.* (*34*). reported that Caucasian hypertensive patients with the *I* allele of *ACE I/D* were more sensitive for hydrochrolthiazide than those with *DD*, no polymorphisms of the RAAS-related genes, including *ACE I/D*, showed a significant correlation with the effect of TZDs in the present study. Furthermore, neither *C825T* of *GNB3* (*5*) nor *Gly460Trp* of *ADD1* (*6*, *34*), which have previously been reported to influence the sensitivity to TZDs, showed a significant correlation with the effect of TZDs in the present study. It is suggested that the reason for the difference between the present findings and previous findings on the participation of the RAAS genes, *GNB3* and *ADD1*, in the effects of TZDs might be the ethnicity of the study subjects. Most studies (*5*, *34*) investigated the participation of the RAAS genes, *GNB3* and *ADD1*, in the effect of TZDs in Caucasians. In contrast, all subjects in the present study

Table 3. TZD- Sensitive Gene Polymorphisms of *TSC C1784T* and *ADRB3 T727C*

SNP	Sex	Genotype	R	NR	χ^2	*p*
TSC C1784T	M+F	CC	40	20	6.052	0.049
		CT	5	9		
		TT	0	1		
		CC	40	20	5.556	0.037
		TT+CT	5	10		
	Odds ratio=4.000, 95% CI=1.204–13.284					
		C allele	85	49	6.168	0.016
		T allele	5	11		
	Odds ratio=3.816, 95% CI=1.253–11.627					
ADRB3 T727C	M+F	CC	1	1	10.649	0.005
		CT	3	11		
		TT	40	18		
		TT	40	18	10.056	0.003
		CC+CT	4	12		
	Odds ratio=6.667, 95% CI=1.889–23.525					
		C allele	5	13	8.533	0.005
		T allele	83	47		
	Odds ratio=4.591, 95% CI=1.541–13.680					

TZD, thiazide diuretics; R, responder; NR, non-responder; SNP, single nucleotide polymorphism; M, male; F, female; CI, confidence interval.

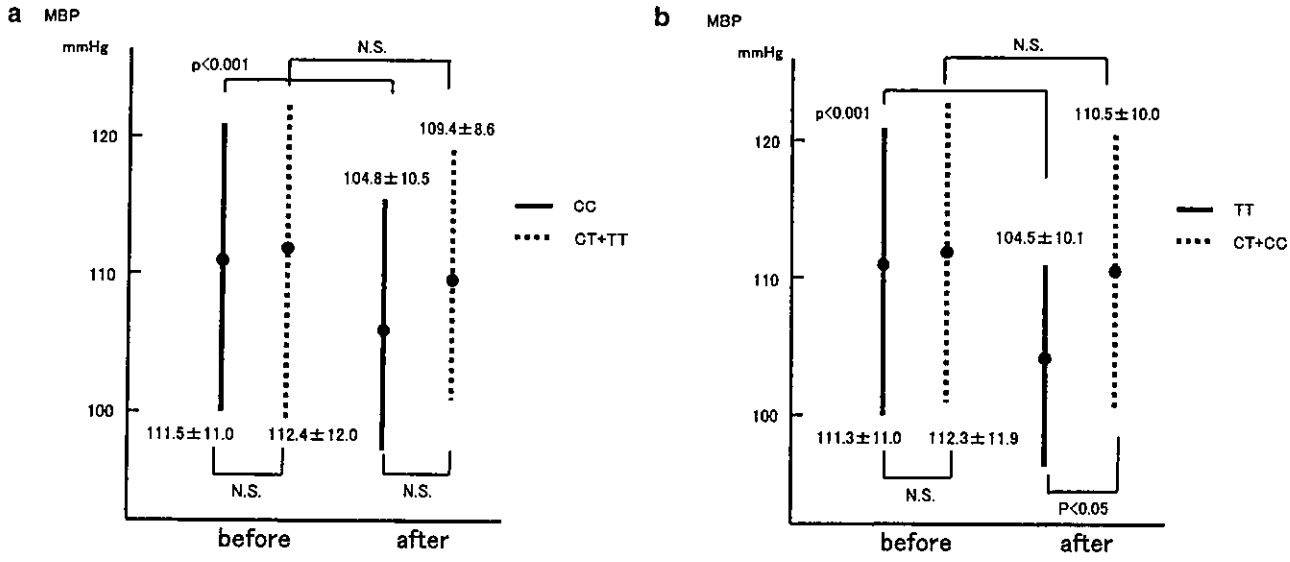

Fig. 3. *a: Comparison of the MBP changes before and after TZD therapy between homozygotes of the major allele CC and the minor homo- and heterozygote TT+CT of* TSC C1784T. *NS, not significant. b: Comparison of MBP changes before and after TZD administration between the homozygotes of the major allele TT and the minor homo- and heterozygote CC+CT of* ADRB3 T727C. *NS, not significant.*

were Japanese. The amount of salt intake in the Japanese population is generally greater than that in Caucasians (*35*). Thus, there is a possibility that Japanese may be more sensitive to TZDs than Caucasians.

The present study revealed a significant correlation between *T727C-Trp64Arg ADRB3* and the effect of TZDs. The β3 adrenergic receptors are mainly distributed in adipose tissues and involved in the regulation of lipolysis and thermogenesis. Regarding the *ADRB3* polymorphism *Trp64Arg*, it has been correlated with obesity (*36*), insulin resistance (*37*) and diabetes (*38*). In contrast, the relation between *Trp64Arg ADRB3* and hypertension is controversial (*24, 39, 40*). Re-

Table 4. Haplotype Distribution in R and NR in Two SNPs of *TSC*

Haplotype		R (n=90)	NR (n=60)	p value
H1	CC	81	47	0.622
H2	CT	5	9	0.094
H3	GC	4	2	>0.999
H4	GT	0	2	0.165

R, responder for thiazide diuretics: NR, non-responder for thiazide diuretics; SNPs, single nucleotide polymorphisms. Haplotypes were shown as combined alleles of genotyped 2 SNPs (*C−213G, C1784T*) of *TSC*.

garding the SNS activity, Shihara *et al.* (*41*) reported that subjects with the homo- and heterozygote of *64Arg ADRB3* had higher responses of the autonomic nerve activities after postural change than those with the wild-type gene. This suggests that *Trp64Arg ADRB3* may play an important role in the autonomic nervous system activities, including the activities of the SNS. To date, however, there has been no evidence that β3 adrenergic receptors exist and function physiologically on sympathetic nerves or the renal tubular system. Therefore, the mechanisms intervening between *ADRB3* gene polymorphism and the effectiveness of TZDs are unclear. Further investigations, including studies on the reflective activation of SNS and the worsening in insulin resistance after TZD administration, will be needed to clarify this interaction.

There is a question as to whether the contributions of *TSC C1784T* and *ADRB3 T727C* to the effects of TZDs are a TZD-specific finding. We tried to investigate the relationship between these 2 SNPs and the antihypertensive effects of an ACEI in 98 patients with EHT by the same study protocol; however, these 2 SNPs did not show any significant correlation to the effect of ACEI (unpublished data). Although investigation of other antihypertensive drugs is necessary, we speculate that the contribution of these 2 SNPs to the effect of TZDs is in fact a TZD-specific finding.

The study limitations include the retrospective design and the small sample size. The study subjects included not only patients receiving monotherapy with TZDs but also those receiving combined therapy with TZDs and other antihypertensive drugs. This issue is not considered to have much influence on the relationship between the 2 SNPs, *TSC C1784T-ADRB3 T727C*, and the effect of TZDs, because the prevalence of patients with monotherapy using TZD and the variation of the kinds of TZDs were not significantly different in between each allele of the two SNPs. However, a prospective and large-scale controlled study using TZDs is needed to confirm the importance of *TSC C1784T* and *ADRB3 T727C* on the antihypertensive effect of TZDs.

Furthermore, the BP level at pretreatment is considered an important factor in the effect of antihypertensive drugs. In the present study, BP before TZD administration was significantly higher in R than in NR. However, the BP level before TZD administration was not significantly different between TZD-sensitive and -insensitive genotypes in both *TSC C1784T* and *ADRB3 T727C-Trp64Arg*, as shown in Fig. 3. It might be possible that the BP response to TZDs was modified by the placebo effect. However, it is unlikely that *TSC C1784T* or *ADRB3 T727C* is involved in the placebo effect. A placebo-controlled prospective trial or ambulatory BP monitoring would help to confirm the significance of these SNPs in the BP-lowering effect of TZDs.

Finally, regarding the statistical approach, the Bonferroni method was not performed in this study even though multiple SNPs were investigated. The criterion for significance is $p<0.001$ (=0.05/48 SNPs) according to the Bonferroni method; however, *TSC C1784T* and *ADRB3 T727C* were associated with the antihypertensive effect of TZDs at $p=$ 0.016–0.049 and 0.003–0.005, respectively (Table 3). Although this might be considered a weak correlation for this kind of genetic research, we consider these two SNPs as prominent candidates relating to the effectiveness of TZDs, because both *TSC* and *ADRB3* were suggested to play an important role in the effectiveness of TZDs in patients with EHT, as we mentioned above.

In conclusion, *TSC C1784T* and *ADRB3 T727C-Trp64Arg*, may be gene polymorphisms susceptible to the antihypertensive effect of TZDs in patients with EHT. Thus, the prediction of BP reduction by TZDs may be possible by evaluating these two SNPs. Since the publication of the JNC 7, TZDs are becoming increasingly important as first-line drugs (*1*). The prediction of the TZD sensitivity of patients may lead to the realization of individualized therapy for hypertension based on genetic background.

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Appendix

The sequences of the allele-specific probes and PCR primers for the genotyping are shown in Table A1 and SNPs of *TSC*, *MLR*, *WNK1*, *WNK4* and *NCX1* are shown in Table A2.

References

1. Chobanian AV, Bakris GL, Black HR, *et al*: The seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 Report. *JAMA* 2003; **289**: 2560–2572.
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Table A1. List of Genotyping Conditions for TaqMan PCR Method

Gene name	SNP	Primer	Final conc. (nmol/l)	Probe	Final conc. (nmol/l)	96-well annealing temp. and cycle no.	384-well annealing temp. and cycle no.
ADD1	*Gly460Trp*	CACACCTTAGTCTTCGACTTGGG	800	Fam-TTCTGCCCTTCCTC-MGB	200		58°C
	(G29071T)	ACAAGATGGCTGAACTCTGGC	800	Vic-TTCTGCCATTCCTC-MGB	200		40
GNB3	*C825T*	CAGACCAGGAGCTGATCTGCTT	800	Fam-CATCACGTCCGTGGC-MGB	200		60°C
		TTGCAGTTGAAGTCGTCGTAGC	800	Vic-ATCACGTCTGTGGCCT-MGB	200		40
TSC	*C−1991A*	CCCTGACAGCTCAAATTTCCAC	800	Fam-CTGCCTCCCTGCAA-MGB	200		58°C
		CTTGTTACCAGAGGTGCCTAAGC	800	Vic-CTGCCTCACTGCAA-MGB	200		40
	C−605T	GCAGAAATGAAATCCACAAGCA	800	FAM-TTTGAAAATCCCTGTCCTG-MGB	228	62°C	58°C
		CATGCACCGATCATTAGATTGG	800	VIC-CTTTGAAAATTCCTGTCCTG-MGB	223	40	40
	C−213G	GGCAGAACACCATTTGATTGTG	800	FAM-CTGGCCCAAAGCCAGCCACTC-TAMRA	256	62°C	60°C
		GAAGAGCCACTCCAGGACTCA	800	VIC-CTGGCCCAAACCCAGCCACTC-TAMRA	282	35	40
	C1784T	CGCAGTGGTGCAGGTCAGT	800	Fam-CAGAGACGCCGTCC-MGB	200		58°C
		AGGTGTCTGCCTTCCTGCTG	800	Vic-TGCAGAGATGCCGTC-MGB	200		40
	A1918G	CTCACCATCACCCCCTTGAC	800	Fam-CTGGTGCCTGCCTCGCCC-TAMRA	200		60°C
		CAGCAGGAAGGCAGACACCT	800	Vic-TGGTGCCCGCCTCGCC-TAMRA	200		40
	A2141T	GCTTCAGTTTCCCCATCTGTACA	800	Fam-AATAGATTAAAGCCTGCCGG-MGB	200		58°C
		GGTGGCTTTTTAGGGAAACACA	800	Vic-AATAGATTAATGCCTGCCGG-MGB	200		40
	C4527A	GATGAACGTAGGTCGCATGGT	800	FAM-TGTCGGTCACGGTGA-MGB	336	60°C	58°C
		GATGGCTGAGATGGAGAGGC	800	VIC-TGTCGGTCAAGGTG-MGB	297	40	40
	T18806C	AGCAGCTCTGGCCTAGAAAGAG	800	FAM-TGGTGCCCTTGGCCCAGG-TAMRA	330	62°C	62°C
		ACGGAGATGATAGCCCCAAAC	800	VIC-CTGGTGCCCTCGGCCCAG-TAMRA	290	35	40
	T29320A	TCACATAGTGCTCTGTCCTGAGTG	800	FAM-TCCCTATCTCCTTGGC-MGB	242	62°C	60°C
		GATCTTGCATTTGCTCCACCTC	800	VIC-CCTATCACCTTGGCC-MGB	201	40	40
	C29372T	GCAAGAGGAGGTGGAGCAAAT	800	FAM-TTCGTAGGCGGCCAG-MGB	117	60°C	58°C
		CCCTCCACACTTACGCCTTTC	800	VIC-TCGTAGGTGGCCAGAT-MGB	254	40	40
	G34372A	GGGATTCCATGAAGTCCACATC	800	FAM-AACCCTCGGGCTGA-MGB	337	62°C	—
		CTGGAAGCCCCAAAACAGAAC	800	VIC-AGAACCCTCAGGCTG-MGB	329	40	—
	C39375T	GAAGCAGAAGGGCCAAAGTTC	800	FAM-ATAGCCCTGGCGATT-MGB	267	58°C	58°C
		GATGCCTGGGACACGTGAG	800	VIC-TAGCCCTGGTGATTC-MGB	84	40	40
MLR	*C−2G*	TTGTGGCTTAGCAAATGCAATT	800	Fam-TTTGTTAGCGATGGAGAC-MGB	602	62°C	
		CAGGGAGACTGTGGTAGCCTTT	800	Vic-ATTTGTTAGGGATGGAGAC-MGB	224	40	
	G538A	GGGCTTTTCTCATGACACATGATA	800	Fam-CTTTTAACAATGGCGCGC-MGB	189		60°C
		CGCCCTTGAGATCATTTATGTCT	800	Vic-TTTAACAACGGCGCGCA-MGB	361		40
NCX1	*T−23690C*	CTCTCCCCACAGGTCATTCTG	800	Fam-ATTTAACTTATAGCAAGGAA-MGB	200		58°C
		GCAGGAATCGTTCTTGCCTAA	800	Vic-TTAACTTACAGCAAGGAA-MGB	200		40
	C−23449A	GAATCTGCAATCCCCATGTGAT	800	Fam-CTCACATTCATGTTTGAG-MGB	200		56°C
		AGAACCACTGCTCTAGGCCAAT	800	Vic-ACTCACATTAATGTTTGAGG-MGB	200		40
	T−23200C	TTCTGAGGTGCAAGGAGGGTT	800	Fam-CCCCCTTTTTGTTGC-MGB	100		56°C
		GGCAGTCACCACGACTGATAGA	800	Vic-CCCCCTCTTTGTTG-MGB	100		40
	T−23181C	GGCAGTCACCACGACTGATAGA	800	Fam-TCCAGGAACCTCAGTTT-MGB	200		56°C
		AGGCTATTTCTTCCATTCCGC	800	Vic-CCAGGAACCTCGGTTT-MGB	200		40
	A−22729C	GCCTGGTGCAGTGTTCCTTTA	800	Fam-ATTATGAGGAAAGTGATTTA-MGB	200		58°C
		GCCCTTTCCAAGAGAAGCATTA	800	Vic-TATGAGGACAGTGATTTA-MGB	200		40
	C−22144G	AAAAGAAAAGTTGCAGCGCCT	800	Fam-CCACAACGCACTGC-MGB	200		56°C
		TTTTTCGATTTCCTGCCGG	800	Vic-CACAAGGCACTGCG-MGB	100		40
	G252581A	AAACAAAGACATACCAGCGAGAAA	800	Fam-CTCTCTCCGTGTCTC-MGB	200		58°C
		AAATTGCTAAAGCTTCAAAGGCA	800	Vic-TCTCTCCATGTCTCC-MGB	200		40
WNK1	*G786A*	GAACTGCAGGTAAAGCCCCAC	800	Fam-TTTGACGGTCCTTTG-MGB	200		58°C
		GAACTCGATCAACTGGCTTCG	800	Vic-TTTATTTGACAGTCCTTTG-MGB	200		40
	C108560T	CTGATGGGACGGTTGACAGTG	800	Fam-TCTTCACAGAATCTCGA-MGB	200		58°C

Table A1. (Continued)

Gene name	SNP	Primer	Final conc. (nmol/l)	Probe	Final conc. (nmol/l)	96-well annealing temp. and cycle no.	384-well annealing temp. and cycle no.
		CCTGTTCATGTTGGGAACCATA	800	Vic-TCTTCATAGAATCTCG-MGB	200		40
	A128177C	GTTGCTCCTGCAGAGCCAGT	800	Fam-AGTAGCACAGACCCAA-MGB	200		58 °C
		TCTACAGAGGAAGCCAAAGTGGT	800	Vic-AGTAGCACAGCCCCA-MGB	200		40
	C133634T	TTGATTTGCTCTTCAGTACGCAG	800	Fam-AGCGTCTCACGGACT-MGB	200		58 °C
		GCACCTACAGACAACAAAGGGAA	800	Vic-AGCGTCTCATGGACT-MGB	200		40
	G135642T	AAAACTTACACCAACCGCAGAAG	800	Fam-CTGTGATCATCTCTG-MGB	200		58 °C
		ATTCAGTCCCAGCAACCTCTAGA	800	Vic-ACTGTGATAATCTCTG-MGB	200		40
	C141114T	TGGGACGATTTCAGGTAAGACAG	800	Fam-ATTCCTTCCTTTGGAGGA-MGB	200		58 °C
		TTGTGTCCCAAATAGGTAGGCA	800	Vic-ATTCCTTCTTTTGGAGGAG-MGB	200		40
	C142763T	ACGACCCACTTTGTTTGCTGTA	800	Fam-CTGAAAACGTCCAACCT-MGB	200		58 °C
		GTCAGACACTGGGCAGCCTAC	800	Vic-CCTGAAAACATCCAACCT-MGB	200		40
WNK4	*C14597T*	CTGGCTGTGATGACTGTGGC	800	Fam-TCCCCTCCCTAGCCT-MGB	200		58 °C
		TGAAGGGCTTTCCTGGCC	800	Vic-TCCCCTCTCTAGCCTG-MGB	200		40
	C14717T	CACAGCTGAGGTGGAGAGTGAG	800	Fam-CTCCACTCTGCACTC-MGB	200		58 °C
		GGAGGTGGTGAGGCCTAGAAA	800	Vic-ACTCCATTCTGCACTC-MGB	200		40
AGT	*A(−20)C**	CTTCTGGCATCTGTCCTTCTGG	250	Direct sequence			64 °C
		CTGGTCTTATGAGAGGGGAGAGG	250				35
	*G(−6)A**	Same as *A(−20)C*		Direct sequence			
ACE	*G12568C*	AGCAGAGGTGAGCTAAGGGCT	667	Fam-CTCAAGGCATTCAA-MGB	200		58 °C
	(I/D)	GGCCATCACATTCGTCAGATCT	667	Vic-CTCAAGCCATTCAA-MGB	200		40
AT1	*A(−153)G*	AACGCTGATCTGATAGTTGACACG	800	Fam-CCGTCAATATCCCGAG-MGB	200		60 °C
		CTCTGTTTTGCATTCCCTCCTC	800	Vic-CCGTCAGTATCCCGA-MGB	200		40
	A1166C	AGAGAACATTCCTCTGCAGCACT	800	Fam-CAAATGAGCATTAGCT-MGB	200		60 °C
		CGGTTCAGTCCACATAATGCAT	800	Vic-CAAATGAGCCTTAGCT-MGB	200		40
CYP11B2	*C(−344)T*	TGGACATTTTCTGCAGTTTTTGA	800	Fam-ATCCAAGGCTCCCTCT-MGB	100		56 °C
		TCCTTTCTCCAGGGCTGAGA	800	Vic-CAAGGCCCCCTCT-MGB	100		40
ADRB1	*G1413C*	TTCTTCAACTGGCTGGGCTAC	800	Fam-CCTTCCAGGGACTGC-MGB	200		58 °C
		GTCTCCGTGGGTCGCGT	800	Vic-CTTCCAGCGACTGCT-MGB	200		40
	A393G	CCGGTAACCTGTCGTCGG	800	Fam-CAGCGAAAGCCCCGA-MGB	200		58 °C
		GATCACCAGCACATTGCCC	800	Vic-AGCGAAGGCCCCGAG-MGB	100		40
ADRB2	*C(−47)T*	CATTGGGTGCCAGCAAGAA	800	Fam-CGCCTCAGCGGGCGGA-TAMRA	100		56 °C
		GAATGAGGCTTCCAGGCGT	800	Vic-CGCCTCAGCAGGCGGACC-TAMRA	100		40
	G2118A	CGCTGAATGAGGCTTCCAG	800	Fam-ACCCAATGGAAGCC-MGB	100		58 °C
		CTGCGTGACGTCGTGGTC	800	Vic-ACCCAATAGAAGCCA-MGB	100		40
	G2151C	CCAGGACGATGAGAGACATGAC	800	Fam-TCCCTTTCCTGCGTGA-MGB	200		58 °C
		CCTTCTTGCTGGCACCCA	800	Vic-TCCCTTTGCTGCGTG-MGB	200		40
ADRB3	*T727C*	CACGTTGGTCATGGTCTGGA	800	Fam-CGGAGTCCAGGCGA-MGB	200		58 °C
		GAGGCAACCTGCTGGTCATC	800	Vic-TCGGAGTCCGGGCG-MGB	200		40
ADRA1A	*T44653C*	TCCAGCCAAGAGTTCAAAAAGG	800	Fam-CAGTGTCTCTGCAGAA-MGB	100		56 °C
		CCAGGGCATGTTTGGAAGACT	800	AGTGTCTCCGCAGAA-MGB	200		40
ADRA1B	*G834A*	CGCACTCCTTGTCATCGTTG	800	Fam-TCCTTCCACCCAAGGA-MGB	200		58 °C
		GTCTTGTCCACCGTCATCTCC	800	Vic-CTCCTTCCATCCAAGGA-MGB	200		40
	G1167A	CAAGATGAACATACCGACCACAA	800	Fam-CCCAACGTCTTAGCT-MGB	200		60 °C
		CAACCCCAGGAGTTCCATAGC	800	Vic-CCCAACGTTTTAGCT-MGB	200		40
ADRA2A	*A3023G*	TCCCCTTCCATTCCCAACTC	800	Fam-TCTCTTTTTAAAGAAAAAT-MGB	200		56 °C
		TTCAACATCAAAACCAAGGCC	800	Vic-TCTTTTTGAAGAAAAAT-MGB	100		40

* The genotyping for *AGT A(−20)C* and *G(−6)* polymorphisms was performed by the direct sequence method. A pair of the PCR primers was 5′-CTTCTGGCATCTGTCCTTCTGG-3′ and 5′-CTGGTCTTATGAGAGGGGAGAGG-3′.

Table A2. List of 130 Polymorphisms and Their Allele Frequency in *TSC*, *MLR*, *WNK1*, *WNK4* and *NCX1* Genes Identified by the Direct Sequence

Gene name	Allele 1/Allele 2 SNPs	Amino acid change	Region	Allele 1 Homo	Hetero	Allele 2 Homo	Total	Allele frequency Allele 1	Allele frequency Allele 2	Flanking sequence	dbSNP ID
TSC	*C−1991A*		promoter	38	0	10	48	0.792	0.208	caccactgcctc[c/a]ctgcaatggctt	
	A−950G		promoter	1	19	21	41	0.256	0.744	tttaatagagac[a/g]gggtttcaccat	
	C−704T		promoter	46	1	0	47	0.989	0.011	cagacagcccgg[c/t]gccacaccctgg	
	C−605T		promoter	37	10	0	47	0.894	0.106	cactttgaaaat[c/t]cctgtcctgttt	
	C−553T		promoter	26	1	0	27	0.981	0.019	agcccccagtca[c/t]gtacccccgct	
	−544delT		promoter	47	1	0	48	0.990	0.010	tcacgtacccccc[t/-]gcttgctcaatc	
	C−213G		promoter	35	8	0	43	0.907	0.093	gggagtggctgg[c/g]tttgggccagcc	
	C−142T		promoter	1	20	22	43	0.256	0.744	gtgttctgcctc[c/t]ggccctgtccgg	
	G−141C		promoter	28	15	0	43	0.826	0.174	tgttctgcctcc[g/c]gccctgtccggg	
	C1784T		intron1	30	17	1	48	0.802	0.198	tggatgcagaga[c/t]gccgtccctagc	
	A1918G	Ala122Ala	exon2	31	17	0	48	0.823	0.177	ggagggcgaggc[a/g]ggcaccagcagc	rs2304479
	A2141T		intron2	0	8	40	48	0.083	0.917	acaatagattaa[a/t]gcctgccgggga	rs2304480
	G2971A		intron2	47	1	0	48	0.990	0.010	tagggcctaggt[g/a]ctcgataccctg	
	C4527A	Thr180Lys	exon4	43	2	0	45	0.978	0.022	tgctgtcggtca[c/a]ggtgacctccat	
	C7479T	Phe341Phe	exon8	38	2	0	40	0.975	0.025	tggcaccttctt[c/t]ggaatgttctcc	
	C14272T		intron10	26	18	3	47	0.745	0.255	ctggctcagccc[c/t]caccgtggagtc	rs3816119
	G14277A		intron10	46	1	0	47	0.989	0.011	tcagccccccacc[g/a]tggagtccctga	
	C14363A	Ala464Ala	exon11	45	2	0	47	0.979	0.021	catcttcggggc[c/a]accctctcctct	
	C14366T	Thr465Thr	exon11	46	1	0	47	0.989	0.011	cttcggggccac[c/t]ctctcctctgcc	rs5801
	G17337A		intron13	44	1	0	45	0.989	0.011	ggggtgggagtg[g/a]gaggcatgggtg	
	T18806C[b]		intron13	6	24	18	48	0.375	0.625	gactggtgcccct[t/c]ggcccagggtgg	rs2304483
	C18850T	Ala569Val	exon14	46	2	0	48	0.979	0.021	acaacaagtggg[c/t]ggcgctgtttgg	
	T20072C	Leu623Pro	exon15	46	1	0	47	0.989	0.011	gctcctacaacc[t/c]ggccctcagcta	
	G20088A	Ser628Ser	exon15	46	1	0	47	0.989	0.011	cctcagctactc[g/a]gtgggcctcaat	
	C20201G		intron15	46	1	0	47	0.989	0.011	gagtttccaagc[c/g]tagacctgtcac	
	G21421A		intron16	20	24	3	47	0.681	0.319	atgggggcccaa[g/a]gggatgcggagc	
	C21500T		intron16	42	2	0	44	0.977	0.023	ccctcttgctgg[c/t]ttctcccccagc	
	C21566G		intron16	43	1	0	44	0.989	0.011	cactttctcccc[c/g]actccttgtgtt	
	A21586G		intron16	43	1	0	44	0.989	0.011	gtgttttcccttt[a/g]tctgggcaaaag	
	C21822T	Ala714Ala	exon17	21	21	3	45	0.700	0.300	ggatgtcattgc[c/t]gaggacctccgc	
	C22682T		intron17	46	1	0	47	0.989	0.011	tcacccctatcc[c/t]ctggcaggccgc	
	C25013T[c]		intron18	23	22	3	48	0.708	0.292	ctgggggagaag[c/t]tggacctcacct	rs3764264
	G27029A		intron20	18	25	4	47	0.649	0.351	ttttcttgtgac[g/a]gtggtgcctgag	
	C27646T[b]		intron20	6	26	15	47	0.404	0.596	aaggggcgttgg[c/t]ggggccctgggc	rs2278490
	T27681C[b]*		intron20	5	23	18	47	0.351	0.628	tggatgcgcggc[t/c]gctggctctgct	rs2278489
	*A27681C**			0	1	—		0.011	—	tggatgcgcggc[a/c]gctggctctgct	
	*T27681A**			—	0	—		—	—	tggatgcgcggc[t/a]gctggctctgct	
	T29320A	Leu849His	exon22	367	5	0	372	0.993	0.007	tcattccctatc[t/a]ccttggccgcaa	
	C29372T[c]	Gly866Gly	exon22	23	22	3	48	0.708	0.292	tgtgttcgtagg[c/t]ggccagattaac	rs5804
	G34262A		intron22	44	1	3	48	0.927	0.073	tctcaagaaaaa[g/a]taataacaataa	
	G34372A[d]	Arg904Gln	exon23	45	3	0	48	0.969	0.031	accagaaccctc[g/a]ggctgagcagta	
	C34588T		intron23	41	3	4	48	0.885	0.115	cacagggcaagg[c/t]ggctgcagcccc	
	T37125C		intron23	46	1	0	47	0.989	0.011	cctcaacccact[t/c]tctcgtccccag	
	C37210T	Asn931Asn	exon24	46	1	0	47	0.989	0.011	ggccactgtcaa[c/t]gagatgcggcgg	
	A37311G[e]		intron24	23	21	3	47	0.713	0.287	acgcgacacatc[a/g]ctgggtcaggga	rs2289117
	G39097A		intron24	29	1	0	30	0.983	0.017	gaggccatagac[g/a]tggtgaaggatt	
	C39119T		intron24	29	1	0	30	0.983	0.017	attgagtgacct[c/t]gatgatatggga	
	C39142T		intron24	40	7	0	47	0.926	0.074	gaagtgaccact[c/t]ggctttctcccg	rs3816118
	G39143A[d]		intron24	44	3	0	47	0.968	0.032	aagtgaccactc[g/a]gctttctcccgc	rs2289116
	C39203T	Ser967Phe	exon25	46	1	0	47	0.989	0.011	tgctggattact[c/t]ccgagacgctgc	