Title: IMPROVED THERAPEUTIC REGIMEN FOR HYPERTENSION

Abstract: The invention relates to improved methods, devices, and kits for identifying and implementing an appropriate treatment regimen for subjects suffering from hypertension.
IMPROVED THERAPEUTIC REGIMEN FOR HYPERTENSION

Cross Reference to Related Applications

This application claims benefit of the priority filing date of United States Provisional Application Serial No. 62/004,460, filed May 29, 2014, the contents of which are specifically incorporated herein by reference in their entirety.

Background

Hypertension (high blood pressure) is one of the most significant preventable contributors to disease and death in the world and represents the most common condition seen in the primary care setting (Kearney et al, *Lancet* 365:217-223 (2005)). According to the American Heart Association, approximately 78 million adults (1 in 3) living in the United States have hypertension with more than 5 million new diagnoses made each year. Of these individuals, 82% are aware they have it, 75% are currently being treated for it, but only 52% have their blood pressure under control (thus, -48% do not have adequate blood pressure control).

Hypertension can lead to myocardial infarction (heart attack), stroke, renal failure, and death if not detected early and treated appropriately. In 2009, high blood pressure was listed as a primary or contributing cause of death in about 350,000 of the approximate 2.4 million U.S. deaths (14% of all deaths). From 1999-2009 the number of deaths attributable to hypertension increased by 44%.

Refractory (or resistant) hypertension is defined as blood pressure that remains above clinical guideline goals in spite of concurrent use of three antihypertensive agents of different classes. Critically, refractory hypertension is noted in approximately 25-30% of all individuals being treated for hypertension. Refractory hypertension is a common clinical problem which contributes to the high levels of morbidity and mortality. In 2009, the direct and indirect economic burden on the United States health care system associated with hypertension was estimated at $51 billion.
Globally, nearly 1 billion individuals have been diagnosed with hypertension, with an estimate of an additional 400 million living with undiagnosed hypertension. Hypertension is the leading cause of premature death and the leading cause of cardiovascular disease worldwide. Similar to the continued upward trend in prevalence as seen in the United States, it is estimated that in 2025 about 1.56 billion adults will be living with hypertension. Because nearly two-thirds of the people living with hypertension worldwide reside in developing countries, providing optimal treatment at the lowest cost is critically important.

Unfortunately, despite a significant impulse in the medical community to move towards an "individualized medicine" approach to patient centered treatment, the current clinical treatment strategy is based on a set algorithm which does not take into account individual patient differences. Rather, physicians are guided to choose a drug (one out of many options) in a given class of drugs and use that specific drug as a "first line therapy" (typically initiating with the diuretic class) and titrate that specific drug of choice to therapeutic dosage regardless of efficacy. It is only after a prolonged course of treatment with that specific class of drug that clinical efficacy is determined (typically three months). At this stage, if clinical guideline goals for blood pressure have not been met, it is often recommended that the patient remain on the "first line therapy" whilst an additional drug from a different class of drugs (typically an Angiotensin converting enzyme inhibitor (ACE inhibitor) or Angiotensin II receptor blocker (ARB)) is added to the pharmacologic regimen. Again, this drug is titrated to recommended therapeutic dosage and another prolonged course of treatment is initiated before clinical efficacy is determined (an additional three months - six months since initiation of treatment). If at this point, clinical guideline goals for blood pressure have not been met, a third drug from a third class of drugs (typically a beta-blocker) is added and the process is repeated (another three months - nine months from initiation of treatment).

Further, if clinical guideline goals have continued to be elusive, the diagnosis of refractory hypertension is added and the process is reinitiated with a different combination of drugs, different classes of drugs, different drug options within a given class of drugs, different dosages, or all of the above. Thus, from the time of initial diagnosis and the start of treatment to the point in which blood pressure
is adequately controlled may take anywhere from three months to well over one year. This trial-and-error standard of care is clearly not optimal.

Summary

The invention relates to improved methods, devices, and kits for identifying and implementing an appropriate treatment regimen for subjects suffering from hypertension. The methods, devices, and kits comprehensively assess common genetic variants in the cardiac, vascular, and renal systems in an effort to improve therapeutic guidance for high blood pressure treatment.

Detection of an individual's genetic variants permits selection appropriate drug classes for that individual. Clinicians can then guide blood pressure therapy using knowledge that is specific to their individual patient, rather than the currently employed "trial-and-error" procedures that are based on population data and use of drugs with the least initial side effects.

One aspect of the invention is a method that includes:

(a) administering a loop diuretic to a subject as a first line therapy, without a beta blocker and without a vasodilator, if the subject's genome comprises a WNK1 nucleic acid with a cytosine at the variable position of rs1 159744 or rs2107614;

(b) administering a hydrochlorothiazide to a subject as a first line therapy, without a beta blocker and without a vasodilator, if the subject's genome comprises an ADD1 nucleic acid with a thymine at the variable position of rs4961, or if the test sample comprises a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927; or

(c) administering a hydrochlorothiazide to a subject as a first line therapy, without a beta blocker and without a vasodilator, if the subject's genome comprises an ADD1 nucleic acid with a thymine at the variable position of rs4961, or if the test sample comprises a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927.

Another aspect of the invention is a method that includes: administering a beta-blocker drug to a subject as a first line therapy, without a diuretic and without a hydrochlorothiazide, if the subject's genome does not comprise:

(a) a WNK1 nucleic acid with a cytosine at the variable position of rs1 159744;
(b) a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;  
(c) an ADD1 nucleic acid with a thymine at the variable position of rs4961; or  
(d) a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927

but the subject's genome does comprise:

1. a CYP2D6 nucleic acid with an adenine at the variable position of Rs3892097;  
2. an ADRB1 nucleic acid with a cytosine at the variable position of rs1801253;  
3. an ADRB1 nucleic acid with an adenine at the variable position of rs1801252;  
4. an ADRB2 nucleic acid with a guanine at the variable position of rs1042714; or  
5. an ADRB2 nucleic acid with a guanine at the variable position of rs1042713.

Another aspect of the invention is a method that includes: administering an angiotensin II receptor blocker to a subject as a first line therapy, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the subject's genome does not comprise:

(a) a WNK1 nucleic acid with a cytosine at the variable position of rsl 159744;  
(b) a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;  
(c) an ADD1 nucleic acid with a thymine at the variable position of rs4961; or  
(d) a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927

but the subject's genome does comprise:

1. a renin nucleic acid with a cytosine at the variable position of rs12750834; or  
2. an AGTIR nucleic acid with a cytosine at the variable position of rs5186.
Another aspect of the invention is a method that includes: administering an ACE inhibitor to a subject without an angiotensin II receptor blocker as a first line therapy, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the subject's genome does not comprise:

- (a) WNK1 nucleic acid with a cytosine at the variable position of rs1 159744;
- (b) a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;
- (c) an ADD1 nucleic acid with a thymine at the variable position of rs4961; or
- (d) a SLC12A3 nucleic acid with a thymine at the variable position of rs1 529927

but the subject's genome does comprise:

1. an ACE nucleic acid with a deletion in rs1 799752; or
2. an AGT nucleic acid with a cytosine at the variable position of rs699.

Another aspect of the invention is a method that includes: administering an amiloride as a first line therapy to a subject without an ACE inhibitor, without an angiotensin II receptor blocker, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the subject's genome does not comprise:

1. a WNK1 nucleic acid with a cytosine at the variable position of rs1 159744;
2. a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;
3. an ADD1 nucleic acid with a thymine at the variable position of rs4961; or
4. a SLC12A3 nucleic acid with a thymine at the variable position of rs1 529927.

but if the subject's genome does comprise a SCNN1A nucleic acid with an adenine at the variable position of rs2228576.

The methods can also include administering a second line therapy drug after administration of the first line therapy for at least 1 month, wherein the
second line therapy drug is selected from the group consisting of diuretic, a beta-blocker, an ACE inhibitor, a vasodilator, and a combination thereof.

Devices, compositions, methods, and kits are also described herein for identifying and implementing an appropriate treatment regimen for subjects suffering from hypertension.

**Description of the Figures**

FIG. 1 is a schematic diagram illustrating the interplay between the heart, blood vessels, and kidney in blood pressure regulation.

FIG. 2 is a schematic diagram illustrating of the types of genes useful for evaluating hypertension, and representative single nucleotide polymorphisms that are correlated with blood pressure drug responses.

FIG. 3A-3B are schematic diagrams illustrating processing of test samples. For example, each subject can collect two swabs. The A swab can collect cell material from the inside of the right cheek, while the B swab can collect cell material from the left cheek. For FIG. 3A, the A swab can be the initial swab entered into the process (from DNA Extraction to Reporting). If the A swab fails, during DNA Yield and Purity Analysis, Genetic analysis, or the PCR QA Assay then the B swab can be entered into the system as illustrated. FIG. 3B shows the same process but without the steps of DNA Stocks Storage and Future Testing if the sample passes Yield and Purity Assays.

FIG. 4 is a schematic diagram illustrating handling of DNA samples.

FIG. 5 is a schematic diagram illustrating processing of the sample after PCR amplification.

FIG. 6 illustrates alignment of sample results to a human reference sequence using the CLC DNA workbench program for creating an alignment file from which the allele call is made and added to the final SNP call report (SEQ ID NOs:82-85).

FIG. 7 is an example of 2% agarose gel used to score the presence or absence of a 288bp ALU by visually examining the gel for either the higher molecular weight band (indicating the presence of the 288bp ALU), the lower molecular weight band (indicating the absence of the 288bp ALU), or both (indicating a heterozygous state).

FIG. 8 is a bar graph of urinary sodium output as a function of genetic variation of SCNN1A.
FIG. 9 is a bar graph of mean arterial blood pressure as a function of genetic variation of SCNN1A.

Detailed Description

Methods, devices, and kits are described herein for selecting individualized hypertension treatment regimens that are more effective than currently available regimens. The methods, devices, and kits include assays for identifying genetic variants in individual subjects that make the individual more or less responsive to specific medications. When the appropriate medication is administered, the subject's blood pressure is appropriately controlled, and side effects are avoided. Genetic variants present in genes such as ADRB1, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptor, renin, Na\(^+\) channels (such as SCNN1A), adducin, sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporters (such as SLC12A3), and/or WNK1 genes are correlated with heightened or reduced responsiveness to various blood pressure medications. Although there are a number of hypertension drugs available on the market, subjects react differently to such drugs. The kits, methods, and devices described herein are useful for detecting which subjects benefit from which therapeutic regimen.

High Blood Pressure (Hypertension)

The development of high blood pressure in humans is the result of one or more of three physiologic maladaptations: 1) elevated cardiac output (liters of blood ejected from the heart per minute) that increases the amount of blood pressing against the vessels, 2) relatively narrow blood vessels that results in increased pressure towards the lumen of the blood vessel due to PoiseuiUe's Law (which describes the inverse relationship between the diameter of a tube (vessel) and the pressure against the walls of the tube (vessel), all else being equal), or 3) increased sodium (Na\(^+\)) absorption in the kidney which results in increased blood volume (and the amount of blood pumped per minute, cardiac output) and subsequently increased outward pressure against the tubes (vessels).

Blood pressure is highly regulated and tightly controlled in humans, such that in the event of a drop in cardiac output, the heart sends a signal to the kidneys (via the proteins atrial natriuretic peptide and brain-type natriuretic peptide, among others) and vessels with the ultimate function of increasing Na\(^+\)
reabsorption to increase plasma volume and vasoconstriction to increase blood pressure. Similarly, if there is a drop in blood pressure, there is an increase in cardiac output (primarily via an immediate increase in heart rate through inhibition of the parasympathetic nervous system) to compensate and an increase in the renin-angiotensin aldosterone system which results in both constriction of blood vessels (which increases blood pressure) and an increase in Na\(^+\) and, therefore, fluid retention in the kidney, which increases plasma volume and can increase blood pressure. Hence, the human body provides redundant functions to maintain blood pressure both in the short-term and in the long-term, by regulating the interplay between the heart, blood vessels, and kidney. FIG. 1 is a schematic diagram illustrating the interplay between the heart, blood vessels, and kidneys involved in regulating blood pressure.

Blood pressure therapy following diagnosis traditionally follows a regimented algorithm based on therapies effective across the general population. Currently, clinicians start a patient who has been diagnosed with high blood pressure on a diuretic (to reduce renal Na\(^+\) reabsorption). If such a diuretic does not work within a period of time, the clinician next tries a vasodilator, and if this is not effective, then a clinician will lastly prescribe a beta-blocker. This trial-and-error process to lower a patient's blood pressure can take several months, is costly, and threatens the health of the patient because the patient's hypertension is frequently not adequately controlled in a timely manner.

Such currently available methods are in stark contrast to the methods, devices, and kits described herein that involve specifically testing an individual's genetic profile and, as illustrated herein, basing therapeutic treatment on the results of such testing. Hence, the methods, devices, and kits described herein for evaluating a blood pressure genetic panel to improve treatment of hypertensive subjects, by quickly identifying more effective medications, thereby avoiding side effects and delays in treatment. Applicants' methods are therefore an improvement over the currently available trial-and-error procedures that frequently result in side effects and delays in effective treatment.

**Ranking of Genotypes that Affect Therapy**

The genotype of a subject can significantly affect the response of the subject to blood pressure medications because certain functional polymorphisms have greater effects on the physiology of a subject than others. The following is
a summary of polymorphisms in order of their impact on blood pressure and which drugs should be employed by subjects with such genetic variations.

1. Subjects with the WNK1 polymorphism defined by rsl 159744 (SEQ ID NO:34, with cytosine at the variable position), benefit more from loop diuretics.

2. Subjects with the WNK1 polymorphism defined by rs2107614 (SEQ ID NO:33, with cytosine at the variable position), benefit more from loop diuretics.

3. Subjects with the ADD1 polymorphism defined by rs4961 (SEQ ID NO:27, with thymine at the variable position), benefit more from hydrochlorothiazides.

4. Subjects with the SLC12A3 polymorphism defined by rsl529927 (SEQ ID NO:30, with thymine at the variable position), benefit more from hydrochlorothiazides.

Any homozygous combination at one or more of the WNK1 Rsl 159744, WNK1 Rs2107614, ADD1 Rs4961, and SLC12A3 rsl 529927 polymorphisms means that diuretics should be the first-line therapy, especially if the patient is heterozygous for polymorphisms in genes responsive to beta-blockers or vasodilators.

5. Subjects with the CYP2D6 polymorphism defined by Rs3892097 (SEQ ID NO:10, with adenine at the variable position), plus the ADRB1 polymorphism defined by rsl801253 (SEQ ID NO:3, with cytosine as the variable nucleotide), plus the ADRB1 polymorphism defined by rsl 801252 (SEQ ID NO:2, with adenine as the variable nucleotide) benefit from beta-blockade classes of drugs.

6. Subjects with the renin polymorphism defined by rsl2750834 (SEQ ID NO:20, with cytosine as the variable nucleotide) plus the AGT1R polymorphism defined by rs5186 (SEQ ID NO:16, with cytosine as the variable nucleotide), which affect responses to angiotensin II receptor blockers.

7. Subjects with the ACE deletion defined by Rsl799752 (SEQ ID NO:35) and the AGT polymorphism defined by rs699 (SEQ ID NO:14, with cytosine as the variable nucleotide) can benefit from the combination of an angiotensin II receptor blocker and an ACE inhibitor.
8. Subjects with the SCNN1A polymorphism defined by rs2228576 (SEQ ID NO:22, with adenine as the variable position) can benefit from administration of amiloride.

9. Subjects with the ADRB2 polymorphism defined by rs1042714 (SEQ ID NO:7, with guanine as the variable nucleotide) can benefit from administration of a non-selective beta-blockade.

10. Subjects with the ADRB2 polymorphism defined by si042713 (SEQ ID NO:6, with guanine as the variable nucleotide) can benefit from administration of a non-selective beta-blockade.

All patients do not respond to same. Some subjects have genotypes that can significantly affect their response to medications. When clinicians employ currently available procedures (diuretic first, then vasodilator, then beta blocker), some patients will benefit but others will not respond or will respond negatively. Hence, some patients would benefit from initial administration of a vasodilator or a beta-blocker, rather than a diuretic.

**Beta-Blocker Responsive Polypeptides and Nucleic Acids**

There are two primary receptors within the heart that influence both heart rate (chronotropic effect) and heart contractility (inotronic effect) (Brodde, *Am J Cardiol* 62:24C-29C (1988), the beta-1 adrenergic receptors (βARv, encoded by the ADRB1 gene) and the beta-2 adrenergic receptors (β2AR, encoded by the ADRB2 gene).

The heart is primary comprised of beta-1 adrenergic receptors, which are located on 80% of the ventricular wall surface, 70% of the atrial wall surface, and 95% of the sino-atrial (SA) node. The atria of the heart receive blood that returns from the body (right atria) of lungs (left atria) whereas the ventricles pump blood to the lungs (right ventricle) and body (left ventricle). The sino-atrial node primarily controls heart rate.

Although heart rate and cardiac contractility are primarily regulated by β1AR, the β2AR also play a role, primarily in cardiac contractility. Stimulation of either β1AR or β2AR can influence heart rate and cardiac contractility through increases in intracellular c-AMP and protein kinase A (PKA) which, ultimately, alter Ca²⁺-channel sensitivity and reduce the threshold needed for an action potential. Therefore, cardiac output (and blood pressure) can be increased.
through increases in $\beta_1$AR and/or $\beta_2$AR activities. If a variant $\beta_1$AR or $\beta_2$AR gene encodes a more functional receptor, cardiac output is increased.

$\beta_1$AR and $\beta_2$AR activities can be modulated through the use of selective (e.g., atenolol and metoprolol) and non-selective (e.g., propranolol and carvedilol) beta-blockers. The selective beta-blockers are selective for inhibiting the PiAR. The non-selective beta-blockers inhibit both PiAR and $\beta_2$AR. Both types of beta-blockers tend to decrease blood pressure through a decrease in heart rate and cardiac contractility, which ultimately results in a decrease in cardiac output. Similarly, the administration of a $\beta_2$AR-agonist (e.g., albuterol sulfate) tends to increase cardiac output and heart rate (Snyder et al, Pharmacotherapy 31:748-756 (2011)). Thus, both PiAR and $\beta_2$AR are important in the regulation of cardiac output.

Just as stimulation of these receptors can elevate cardiac output and increase blood pressure, so too can genetic variation of the genes that encode PiAR and $\beta_2$AR (ADRB1 and ADRB2) elevate receptor activity and increase blood pressure. Conversely, some ADRB1 and ADRB2 genetic variants encode receptors with reduced activity. In addition, some ADRB1 and ADRB2 genetic variants exhibit reduced, or enhanced, responsiveness to blood pressure medications such as beta-blockers. Not all individuals respond similarly to beta-blockade, despite similar clinical and environmental conditions. As described herein, the effectiveness of beta-blockers is dependent to some extent upon the genetic make-up of the subjects to which the beta-blockers are administered.

Sequences for various adrenergic receptors are available, for example, from the National Center for Biotechnology Information (see website at ncbi.nlm.nih.gov).

For example, a full length human ADRB1 cDNA nucleotide sequence is available from the database maintained by the National Center for Biotechnology Information (see website at ncbi.nlm.nih.gov), which has accession number NM_00064 (GI: 110349783), and is shown below as SEQ ID NO:1.

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1  GCACCACGCC GCCCCGGCTT CTGGGGTGT T CCCAACCAC
41  GGGCCACGCC TGCCACACCC CCCGCCCCCG GCCCTCGCAG
81  CTCGCGATGG GCGCGGGGT GCTCGTCCTG GGCGCCTCCG
121  AGCCCGGTAA CCTGCTGCTG GCGCACCACC TCCCGGACCG
161  CGCGCGCCACC GCGCGCGCGC TGCTGCTGCC CGCGTCGCCG
201  CCCGCGCTCGT TGCTGCTCCG CGCCAGCAGA AGCCCGGACG
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| 241 | CGCTGTCTCA | GCAGTGGAAC | GCGGCGATGG | GTCTGCTGAT |
| 281 | GGCGCTCATC | GTGCTGCTCA | TCGTGGCGGG | CAATGTGCTG |
| 321 | GTGATCGTGG | CCATCGCCAA | GACGCCGCGG | CTGCAGACGC |
| 361 | TCGGCGGCGG | GCCCATCACCT | CGCCCTTCCG | CTACCAGAGC |
| 401 | GGTCATGGGG | CTGCTGGTGG | TGCCGTTCGG | GGCCACCATC |
| 441 | GTGGTGTGGG | GCCGCTGGGA | GTACGGCTCC | TTCTTCTGCG |
| 481 | AGCTGTGGAC | CTGCTGCTCA | TCGTGGCGGG | TGACGGCCAG |
| 521 | CATCGAGACC | CTGTGTGTCA | TTGCCCTGGA | CCGCTACCTC |
| 561 | GCCATCACCT | CTGCATACCTG | TTACCGAGGG | CTGCTGACGC |
| 601 | GCCGCGGGGC | GCGGGGCCTC | GTGTGCACCG | TGTGGGCCAT |
| 641 | CTCGCGCGGC | CTGGCCCGCTG | TGCCCATCCT | CATGCACTGG |
| 681 | TGGCGGGCGG | AGAGCGACGA | GGCGCGCCGC | TGCTACAACG |
| 721 | ACCCGCGCGC | CTGCGACTTC | GTCACCAACC | GGGCCTACGC |
| 761 | CATCGGCTCG | TCGGTAGTCT | CCTTCTACGT | GCCCCTGTGC |
| 801 | ATCATGGCCT | TCGTGTACCT | GCGGGTGTTC | CGCGAGGCCC |
| 841 | AGAAGCAGGT | GAAGAAGATC | GACAGCTGCG | AGCGCCGTTT |
| 881 | CTCGCGCGGC | CCAGCGCGGC | CGCCCTCGCC | CTGCGCCCTCG |
| 921 | CATCGGCTCG | TCGGTAGTCT | CCTTCTACGT | GCCCCTGTGC |
| 961 | GCCGCGGCGG | GCCGCCCGGA | GCCGCCCGGA | GCCGCCCGCG |
| 1001 | GCCGCCCGCG | CGCCGCCACC | GCCGCCCTGG | CCAACGGGCG |
| 1041 | GCCGCGGCGG | GCCGCCCGCG | GCCGCCCGGA | GCCGCCCGCG |
| 1081 | TCTTCACGCT | CTGCTGCTCA | TCGTGGCGGG | CAATGTGCTG |
| 1121 | GTGATCGTGG | CCATCGCCAA | GACGCCGCGG | CTGCAGACGC |
| 1161 | TCGTGTCTCTC | CTAATTCTGC | GGCATACCGG | AACAGCGGCT |
| 1201 | ATCATGGCCT | TCGTGTACCT | GCGGGTGTTC | CGCGAGGCCC |
| 1241 | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC |
| 1281 | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC |
| 1321 | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC |
| 1361 | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC |
| 1401 | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC |
| 1441 | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC |
| 1481 | GCCGCGCGGC | GCCGCGCGGC | GCCGCGCGGC | GCCGCGCGGC |
| 1521 | GCCGCGCGGC | GCCGCGCGGC | GCCGCGCGGC | GCCGCGCGGC |
| 1561 | GCCGCGCGGC | GCCGCGCGGC | GCCGCGCGGC | GCCGCGCGGC |
| 1601 | GTGATCGTGG | CCATCGCCAA | GACGCCGCGG | CTGCAGACGC |
| 1641 | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC | GCCGCCCGGC |
| 1681 | TTTGGGAAGG | GATGGGAAGG | TGGCCTGCTG | ATGGGCTTCT |
| 1721 | TGGTTTTTTT | TTTCTTTTTT | TTTCTTTTTT | CTTCTTTTTT |
| 1741 | TTTTTTTTTT | TTTTTTTTTT | TTTTTTTTTT | CTTCTTTTTT |
| 1801 | GTGATGTGCTG | GTGATGCACT | TTAGATTTT | TTTCCCCAC |
| 1841 | CAGGTGTGGTT | TGCACTGTC | TCGAGAGGG | CGGAGGAAAA |
| 1881 | GATGGGTGTTG | GGAGAAGGAG | GAGGAAGCAT | TAGGAGGGA |
| 1921 | TTTAAATCGA | TCATCGTGGC | TCCATACCT | TTCCCCGGA |
| 1961 | CAGGAGCACCT | TACAGCTGCA | GAGAGAAGG | ATAGAGAGT |
| 2001 | GATGGGTGTTG | GGAGAAGGAG | GAGGAAGCAT | TAGGAGGGA |
| 2041 | TTTTTATTTTC | TAAGTAATGA | TTTCTGTGCT | TATGAGAAGC |
| 2081 | AAGAGAAGAG | ATGGAGGCAA | AAATAAAAAA | AATCAGCTTT |
| 2121 | CAAGAAATGT | TAAGCTTCTC | TTGGACAAGA | CCCACCTTG |
| 2161 | CTTCTTCTCT | TGAGGGCAAA | CCCGCTGTGC | CCCGCGCGC |
| 2201 | TGGGTGTTTGA | GGCTAGGAGA | TTCTACTCCT | ACAGTGTCG |
| 2241 | TTTGCACACG | AGATAGAAAG | ACTTTTTTAT | ATAAACAGC |
The rs1801252 single nucleotide polymorphism (SNP) is present in the ADRBI gene, where the variable nucleotide at about position 231 (underlined) can be adenine in some individuals and guanine in others. The rs1801252 sequence (SEQ ID NO:2) is shown below, where the underlined A/G is the SNP.

CTCGTTGCTGCCTCCCGCCAGCGAA [A/G] GCCCCGAGCCGCTGTCTCAGCAGT

The rs1801253 single nucleotide polymorphism (SNP) is also present in the ADRBI gene, where the variable nucleotide at about position 1251 (underlined) can be guanine in some individuals and cytosine in others. The rs1801253 sequence (SEQ ID NO:3) is shown below, where the underlined C/G is the SNP.

CCCCGACTTCCGCAAGGCCTTCCAG [C/G] GACTGCTCTGCTGCGCGCGCAGGG

The βAR polypeptide encoded by the ADRB1 cDNA with SEQ ID NO:1 has the following sequence (SEQ ID NO:4).

1 MGAGVLVLGA SEPGNLSSAA PLPDGAATTA RLLVPASPPA
41 SLLPPASESP EPLSQWTAG MGLLMALIVL LIVAGNVLVI
81 VAIAKTPRLQ TLTLNFIMSL ASDLVMGLL VVFPGATIVV
121 WGRWEYGSFF CELWTSVDVL CVTASIELTC VIALDRYLA
161 TSPFRYQSSL TRARARGLVC TVWAISALVS FLPIMHWWR
201 AESDEARRCY NDKKCCDFVT NRAYAIASSV VSFYVPLCIM
241 AFVYLRVRE AKQVKKIDS CERRFLGGA PAPPSPPSPV
281 PAPAPPSPGP RPAAAAAATAP LANGRAKRR PSLVALREQ
321 KALKTLGIIM GVFTLCWLPF FLAVNKVKAHF RELVPDRLFV
361 FFNWLGYANS AFWPIYCRS PDFRKAFQGL LCCARRAARR
401 RHATHGDRPR ASGCLARPGP PPSPSPSPV DDDDVVGATP
441 PARLLEPWAG CNGGAAADSD SSLDEPCRPQ FASESKV
Note that the underlined amino acid at position 49 is serine because some individuals have SEQ ID NO:1 or 2, where the variable nucleotide at about position 231 of SEQ ID NO:1 is adenine. However, position 49 of SEQ ID NO:4 can be glycine in some individuals because those individual have guanine at nucleotide position 231 in SEQ ID NO:1.

Note also that the glycine at position 389 is an arginine (instead of glycine) as shown for SEQ ID NO:4 when position 1251 of SEQ ID NO:1 is a cytosine.

Individuals with serine at βiAR amino acid position 49 and/or arginine at position 389 are more responsive to beta-blockers than those with glycines at these positions. Hence, for example, an individual who expresses the βiAR polypeptide with SEQ ID NO:4, will be more responsive to beta-blockers than an individual who expresses the βiAR polypeptide with glycines at both positions 49 and 389.

A full length human ADRB2 cDNA nucleotide sequence is available from the database maintained by the National Center for Biotechnology Information (see website at ncbi.nlm.nih.gov), which has accession number NM_000024 (GL283483994), and is shown below as SEQ ID NO:5.

1  GCACATAACG GGCAGAACGC ACTGCGAAGC GGGCTCTTCA
  4  GAGGCCGGGC TGGAACTGTC AGGCACCGGC AGCCCTAGGC
  8 1 ACCCGCAAGG CTGAGTTGTC AGGACCGATC CCCACCACAC
 12 1 CCACACCACA GGGCCTGAAAT AGGGCTTCCAG GGCGTCGGCT
 16 1 CGGGCCGCCC AGAGCCCGGC GTGGTGGTCC CCGGCTAGG
 20  1 CGCCCCAGGC CGATGGCCTC ACCGCGCAGA CTGGCGCAGA
 24 1 TGGGGCAACC GGAGAACGGG AGGCGCTTCT TGCTGGACC
 28 1 CAATAGAAGC CATGGCCGCG ACCAGAGAGC ACGAGCGCAA
 32 1 AGGGACGAGG TGTGGGTTGCT GGGCATGGGC ATCGTCATGT
 36 1 CTCTCATGCT CTTGGAACCT GTTTTGGGCA ATGTCGTTTC
 40  1 CATCACAACC ATGGCAAAGT TCGAGCGCTC GCAGCGCTC
 44 1 ACCAATCTCT TCATCATCTC ACTGGCCTGT GCTGTATGCG
 48 1 TCACTGGGCCT GGCAGTGGCT CCGCTGAGG CCGCCTATAT
 52 1 TCTTATGAAA ATGGAGACTTT TTGGCAAATTC TGTTGCGAG
 56 1 TTTTTGAGACTC CCATGCTATG CTGGTGCGTC CCAGCGAGCA
 60 1 TTGGAGTTCC CGTGGTGATCT GCAGGATGTC GCATCTTTGC
 64 1 CATATTCTCA CCTTCAAAGT ACCAGAGCT GCTGACAAAG
 68 1 AATAGCGCGC GGGTGATCAT TCTGATGGTG TGGATGTTGT
 72 1 CAGGCCCTAC CTCTCTCTTG CCCATTCAGA TGCACTGTTA
 76 1 CCGGGCCACC ACCAGGAGG CCATCACTAG CTATGCGCAA
 80 1 GAGACCTGCT GTGACTCTCTT CCAAGACCA GCTATGCGCA
 84 1 TTGCGCTCTC ATCGTGTCCT TTCTACGTTC CCCTGGTGAT
881 CATGGTCTTC GTCTACTCCA GGGTCTTTCA GGAGGCCAAA
921 AGGCAGCTCC AGAAGATTGA CAAATCTGAG GGCCGCTTCC
9 6 1 ATGTCCAGAA ... i s shown below, where the underlined C/G
i s the SNP.
TGCGCCGGACCACGACGTCACGCAG [ C/G] AAAGGGACGAGGTGTGGGTGGTGG
G .

The rs1042713 single nucleotide polymorphism (SNP) is present in the
ADRB2 gene, where the variable nucleotide at about position 285 (underlined)
can be in adenine some individuals and guanine in others. The rs1042713
sequence (SEQ ID NO:6) is shown below, where the underlined A/G is the SNP.
35 CAGCGCCTTCTTTGCTGGCACCCAAT [A/G] GAAGCCATGCGCCGGACCACGAGC
T .

The rs1042714 single nucleotide polymorphism (SNP) is also present in
the ADRB2 gene, where the variable nucleotide at about position 318
(underlined) can be cytosine in some individuals and guanine in others. The
rs1042714 sequence (SEQ ID NO:7) is shown below, where the underlined C/G
is the SNP.

TGCGCCGGACCACGACGTCACGCAG [C/G] AAAGGGACGAGGTGTGGGTGGTG
G .
The β2AR polypeptide encoded by the ADRB2 cDNA with SEQ ID NO:5 has the following sequence (SEQ ID NO:8).

```
1  MGQPNGNSAF LLAPNRSAP DHDDTGRDDE WVVVMGIVM
41  SLVLALIVFG NVLVIATAIK FERLQVTNY FITSLACDDL
5  VMGLAVVPFG AAHILMKMT FGNFWCEFWT SIDVLCTVAS
121  IETLCVIAVD RYFAITSPFK YQSLTIKNKA RVIIIMVVIV
161  SGLTSFLPIQ MH AirwaysQE AINCYANETC DCFTNQAAYA
201  IASSIVSFYY PVLVMVFVYS RVFQEAKRQL QKIDKSEGRF
241  HVQLNLSQEVE DGRTGHGLRR SSKFCLKEHK ALKTLGI IMG
281  TFTLCWLPPF IVNIVHVIQD NLIRKEVYIL LNWIGYVNSG
321  FNPLIYCRSP DFRIAFQELL CLRSSLKAY GNGYSSNGNT
361  GEQSGYHEVEQ EKENKLCCED LPGTEDFVGH QGTVPSDNID
401  SQGRNCSTND SLL
```

Note that the underlined arginine at position 16 of SEQ ID NO:8 is arginine because some individuals have nucleotide sequence SEQ ID NO:5, where the nucleotide at about position 285 is adenine. However, position 16 of SEQ ID NO:8 can be glycine in some individuals because those individuals have guanine at nucleotide position 285 in SEQ ID NO:5.

Note also that the glutamine at position 27 of SEQ ID NO:8 is a glutamic acid when position 318 of nucleotide sequence SEQ ID NO:5 is a guanine.

Individuals with glycine at position 16 and/or glutamic acid at β2AR position 27 are more responsive to beta-blockers than those with arginine and glutamine, respectively, at these positions. Hence, for example, an individual who expresses the β2AR polypeptide with SEQ ID NO:5, will be more responsive to beta-blockers than an individual who expresses the β2AR polypeptide with arginine and glutamine at positions 16 and 27.

The gene that encodes cytochrome P450 2D6 (CYP2D6) has been shown to alter the metabolism of the drugs in the beta-blocker class. This alteration in drug metabolism can alter the amount of bioavailable drug. Poor drug metabolizers tend to have more drugs available in the body for longer and will, therefore, have a greater response to therapy. In contrast, active metabolizers of a drug will have less of the drug available in their system and will respond poorly to therapy.

Because of the importance of CYP2D6 on beta-blocker metabolism, this gene is a useful marker of responsive to beta-blocker therapy.

A full length human CYP2D6 cDNA nucleotide sequence is available from the database maintained by the National Center for Biotechnology
Information (see website at ncbi.nlm.nih.gov), which has accession number NM_000106.5 (GL3925 13720), and is shown below as SEQ ID NO:9.

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The rs3892097 single nucleotide polymorphism (SNP) is present in the CYP2D6 gene, where the variable nucleotide at a splice site at about position 595 in SEQ ID NO:9 (underlined), which can be in adenine some individuals and guanine in others.
The rs3892097 sequence (SEQ ID NO: 10) of the CYP2D6 gene is shown below, where the underlined A/G is the SNP.

CCCTTACCGCATCTCCACCCCCA [A/G] GACGCCCTTTTCGCCCAACGGTCT.

Because the SNP occurs near a splice site, the sequences to the left of the SNP site in SEQ ID NO: 10 do not appear in the SEQ ID NO: 9 nucleotide CYP2D6 cDNA sequence.

The cytochrome P450 2D6 polypeptide encoded by the CYP2D6 cDNA with SEQ ID NO: 9 has the following sequence (SEQ ID NO: 1).

1 MGLEALVPLA VIVAIFLLLV DLMHRQRQRA ARYPGPPLPL
10 PGLGNLLHVD FQNTPYCFDQ LRRRFGVDFS LQLAWTPVVF
20 LNGLAAYREA LTHGEDTAD RRPVPTQIL GFGPRSGQVF
30 LARYGPAWRE QRRFSVSTLR NLGLGKSLE QNWTEAAACL
40 CAANHSGR PFRPNGLLDK AVSNVIALSIT CGRRFEDDDP
50 RFRLDDLQEG EGLKEESGFL REVNAVPVL LHI PALAGKV
60 LRFQKAFLTQ LDELITEHRM TDPAQPRPD LTERAFLAEME
70 KAKNPESSF NDENLRIVVA DLFSAQMTTT STTLAWGLLL
80 MILHPDVQRR VQOEIDDVIG QVRRPEMDQ AQMPYTTAVI
90 HEVQRFGDIV PLGVTHMTSR DIVEQGRFIP KGTTLITNLS
100 RACGLPMLAR MELFLLFSVSL LQHFSFSVPT GQPRFSHHGV
110 FAFLVSPSRY ELCAVPR

Note that the underlined glycine at position 169 of SEQ ID NO: 11 is glycine because some individuals have nucleotide sequence SEQ ID NO: 9, where the nucleotide at about position 595 is guanine. However, position 169 of SEQ ID NO: 11 can be arginine in some individuals because those individuals have adenine at nucleotide position 295 in SEQ ID NO: 9.

A patient with that is homozygous for adenine (AA) at the rs3892097 variable site will express CYP2D6 with arginine at position 169 and will not metabolize metoprolol and propranolol as quickly as those with guanine (glycine). Hence, homozygous individuals with adenine (AA) at the rs3892097 variable site have higher plasma levels of metoprolol and propranolol after taking these drugs than subjects that are not homozygous for adenine (AA) at the rs3892097 variable site. Homozygous individuals with adenine (AA) at the rs3892097 variable site would respond more normally to atenolol and carvedilol, which do not require CYP2D6 for their metabolism.
Vasodilation Therapy

Dilation of blood vessels results in decreases in blood pressure, whereas constriction of blood vessels results in increases in blood pressure. The blood vessels are controlled through local neural signaling that is largely under parasympathetic control, and circulating hormones that are largely under sympathetic control, as well as other circulating proteins. Blood pressure increases following stimulation of the angiotensin receptors, which results in vasoconstriction. Angiotensin receptors are stimulated by angiotensin II, which is converted from angiotensin I through the angiotensin converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor and actively inhibits bradykinin, which is a potent vasodilator.

Therefore, angiotensin converting enzyme is a common target of blood pressure therapy. ACE inhibitors such as lisinopril promote vasodilation which, ultimately, reduces the bioavailability of angiotensin-II. Similarly, angiotensin II receptor antagonists such as losartan act as competitive inhibitors, which decrease the number of receptors that are available to bind to angiotensin-II. Despite the method used for promoting vasodilation (through reductions in ACE or receptor inhibition) the end result, on average in the population, is vasodilation which results in a drop in blood pressure due to the inverse relationship between the size of the vessel and the pressure exerted on the vessel, all else being equal. Despite this benefit there is a "bell-curve" response to these therapies in humans. Not all individuals are responsive to vasodilator therapy.

Several functional polymorphisms of the genes that encode for ACE and angiotensin-II receptors exist, which can affect how a subject responds to vasodilation.

Examples of functional ACE polymorphisms include the insertion or deletion polymorphisms such as a 287 base pair fragment (Ulgen et al, Coron Artery Dis 18:153-157 (2007)). The rsl799752 polymorphism is an insertion/deletion in an intron of the ACE gene, and with the sequence (SEQ ID NO: 12) shown below, where sequences in the bracket are the insertion / deletion. TCCCAT TTCTCTAGACCTGCTGCC  TGAGACGGAGTCTCGCTCTGTCGCC  [ ATACAGTCCTTATATGAGTAAGGCT ]  ATACAGTCCTTATATGAGTAAGGCT .

The deletion removes the bracketed nucleic acid segment so that the rsl 799752 polymorphism will have the following sequence (SEQ ID NO:35).
Research indicates that such an ACE deletion polymorphism results in higher ACE plasma levels and greater reduction in ejection fraction in patients following myocardial infraction (likely from elevations in blood pressure).

(5) McNamara et al, *J Am Coll Cardiol* 44:2019-2026 (2004); Pilati et al, *Congest Heart Fail* 10:87-93 (2004). In addition, patients with the deletion polymorphism are more likely to have left-ventricular hypertrophy when compared to patients with the insertion polymorphism (left-ventricular hypertrophy results secondary to prolonged exposure to high blood pressure).

Subjects with the deletion polymorphism would therefore be most responsive to ACE-inhibition or angiotensin-II receptor inhibition.

At least one functional variant of angiotensin has been found in humans: a cytosine to threonine substitution at nucleotide 4072 (Pilbrow et al, *Hypertension* 49:322-327 (2007); Tang et al, *Am Heart J* 143:854-860 (2002)).

Human angiotensinogen is expressed from the AGT gene. A cDNA nucleotide sequence for human angiotensinogen is provided below as SEQ ID NO: 13 (accession number NM_000029.3 GI: 188595658, from the NCBI database).

```
1 ATCCCATGAG GGGCAGCAG GCACGAGAAGT AGGCACCCCG
41 TTGCCTAAGG AAGACTCTCC CTGTCCCTCT GCCCTCTGCA
81 CCTCCGGCCT GCATGGTCCCT GTCCCTCTTGG GGGGATCAT
121 CTCCCAGGGA TGGTTGAGA GCCTGCTGTT GTGGATCCCA
161 GGCTGCCACA CACCTCAGGG AATGTCGCTGG TTTCTCGGAA
201 CTTTGGCCCT GACCTGCTCA AAATGGCCAT AATGTGTAAC
241 TCGATGCTGC ATCGGTCAAG TCGTTGACAG AGTGAACACTC
281 TGAATCACCT TGACTACAGT AGAAGAGTG ATGCCAGGTCT
321 GAGTTGCTCC TGTGCGCTCT GTCTTGGTCCCA AGCTGGATG
361 GTCTGGCCA AATGCATGAC CTCCTCCCTC AGCTTGGAC
401 GAGCAAGGCT GGCAGCAAGT CACTCAGGTG GCTCCCACAC
441 TAAATAGGGA ATCGTGAAG GCGGGGGGAG AAGACGTGCC
481 TGTGTTTCTG TGCATCAGCT AGAAGGTAT GGGAAGACGA
521 GACCCACAGT CTGAGATCGG TCCTGCGCGA GTGGGATGTA
561 GGGCCACTC CTCGGCCTTC CTGGGCTTGG GTGGCCTGGC
601 TCAGAAGTCA GCAGGGGAAG TACCCATCCCA CACCTGGTGC
641 ATCCAAATGT AGAAGTACCT TGAGACGGT GCAAAGGCCA
681 ATGCCCGGAG GCCAAAGACAG CACCACCTCA TACCTGGCTC
721 ATTAACAGGCG AAGACATCCT GTCGATGAG AAGCCCTCA
761 CAGCAACAGC CTGGTGCTAGT CGTGCGAAAA CTGAAGACCG
801 AAGACAAAGT GAGGCCCCGA ATGGTGGGGA TGGTGGCCAA
841 TCTCTTGGGC TCCCCATAT AATGCAATCG CAGTGACGTA
881 TTCGGCGTG GCTGACGGC CAAGCCCTTC TCCCCACCAGG
921 CTGTCTTGGC CACCAGGGCG GCTCTCTATGC TTGGAGCCCT
961 GGACCACACG GCTGACGGCG TACAGGCAAT CCTGGGTGTT
1001 CCTGAGAAGG ACAAGAACCT CACCTGGCCG CTGGGATGCG
```
The rs699 single nucleotide polymorphism (SNP) is present in the AGT gene, where the variable nucleotide is at about position 1311 in SEQ ID NO: 13 (underlined), which can be in thymine some individuals and cytosine in others. The rs699 sequence (SEQ ID NO: 14) is shown below, where the underlined C/T is the SNP.

GGATGGAAGACTGGCTGCTCCCTG AiC/Tl GGGAGCCAGTGTGGACAG
CACCCTG.
The human angiotensinogen polypeptide encoded by the AGT cDNA with SEQ ID NO: 13 has the following sequence (SEQ ID NO: 15).

```
1  MRKRAPQSEM APAGVSLRAT ILCLLAWAGL AAGDRVYIHP
41  FHLVHNEST CEQLAKANAG KPKDPTFIPA PIQAKTSPVD
5  EKALQDQLVL VAAKLDTEDK LRAAMVGMLA NFGFRIYGM
121 HSELWGVVHG ATVLSPTAVF GTLASLYLGA LDHTADRLOA
161 ILGVPWKDKN CTSRLDAHKV LSALQAVQQL LVAQGRADSQ
201 AQLLLSTVVG VFTAPGLHLK QPFVQGLALY TPVVLPRSLD
241 FTELDVAAEK IDRFMQAVTG WKTGCSLMGA SYDSTLAFNT
281 YVHFQGMKMG FSLAEPQEF WVDNSTSVSV PMLSGMTFQ
321 HWSDIQDNFS VTQVPFTESA CLLIIQPHYA SDLKDVEGLT
361 FQQSNLMNWMK KLSPTIHLT MPQVLQGSCS DLQDLLAQA
401 LPAILHTELN LQKLSNDRIR VGEVLSIFF LVAQGRADSQ
441 ESTQQLNKPE VLEVTNLNPFL FAVDQSAT ALHFLGRVAN
481 PLSTA
```

Note that the underlined methionine at position 268 of SEQ ID NO: 15 is methionine because some individuals have nucleotide sequence SEQ ID NO: 13, where the nucleotide at about position 131 is thymine. However, position 268 of SEQ ID NO: 15 can be threonine in some individuals because those individuals have cytosine at nucleotide position 131 in SEQ ID NO: 13.

The threonine polymorphism of angiotensin results in higher angiotensin levels and higher resting blood pressure values. Therefore, patients with the threonine genetic variant will benefit primarily from an ACE inhibitor (preventing the higher levels of angiotensin I to angiotensin II) or an angiotensin receptor inhibitor.

An example of a functional polymorphism of an angiotensin II receptor type-1 involves an adenine to cytosine substitution at nucleotide 1166 (Miller et al. Kidney Int 56:2173-2180 (1999); Baudin, Pharmacogenomics 3:65-73 (2002)). Human angiotensin II receptor type-1 is expressed from the AGT1R gene. One example of an AGT1R single nucleotide polymorphism is the so-called A1 166->C polymorphism, which is in the 3’ untranslated region of the AGT1R gene. This A1 166->C polymorphism is also identified as the rs5186 single nucleotide polymorphism (SNP), which has the following sequence (SEQ ID NO: 16) where the underlined A/C is the variable SNP site.

```
TGCAGCACTTCACTACCAAATGAGC [A/C] TTAGCTACTTTTCAGAATTGAAGG
```
A portion of a 3' untranslated region of the AGT1R gene with NCBI accession number NG_008468.1 (GI: 198041751) is shown below (SEQ IDNO:17) that contains the rs5186 SNP with the variant nucleotide (adenine) identified below in bold and with underlining.

\[
\begin{align*}
48961 & \text{ATTCAACTAG GCACTGCTAGA ATTGAGATA} \\
49001 & \text{TTCGATGATTG TATGAGTTTA} \\
49041 & \text{TATTTCCACA TGCTTAATCT TGGCTTCCTG} \\
49081 & \text{GGGAAATAAT TTTAAGCTAT TTTTCCTCGT} \\
49121 & \text{ATATTCCAAA AAAAGCTCAAC ACCCTACAC} \\
49181 & \text{AAAAATGAGG ACCCTTTTCT ACCCTCAAGT} \\
49201 & \text{AGCTCATTTAG ACCCTACAC TTTTCCTCAT} \\
49241 & \text{AGTGACATGT ACGCTTTTCT AGTAGGAGAA} \\
49301 & \text{AAAGAGGAGG AGAGAGACAA GGGCGGTGAT} \\
49321 & \text{CCAAAAATGA ATTAGCCTAC TTTAAGCTAA} \\
49361 & \text{ATGTCATTTAG TGGTGATGAT ACACCAAGAA} \\
49401 & \text{AAGGAGGAGG AGAGAGACAA GGGCGGTGAT} \\
49441 & \text{GCCATTTATG GCACTGCTAC TTTAAGCTAA} \\
49481 & \text{GAATAGCAGA AGACCAAGGA TGGCTTTTCT} \\
49521 & \text{TTACTGACAG AATAGCAGA TTTAAGCTAA} \\
49561 & \text{TCTTCCTTGA TAAAAGGTATG AAAAAATTAT} \\
49601 & \text{AAATTCGCTA GAGGAGGAGGA AGATGCTTGT} \\
49641 & \text{TATTCTGTCC ATGTCT CCTTCAAGG AATAGCAGA} \\
\end{align*}
\]

This polymorphism has been shown to influence resting blood pressure values which suggest which patients may benefit more from angiotensin-II receptor inhibition. Specifically, patients with the C variant of the angiotensin receptor type I tend to demonstrate higher resting blood pressure values, have more detrimental cardiovascular events, and have a greater chance of developing high blood pressure during pregnancy, when compared to the A variant. Subjects with the C variant will therefore be more responsive to angiotensin receptor blockers.

A cDNA sequence for human angiotensin II receptor is provided in the NCBI database as accession number X65699.1 (GI:5 10983), which has the following sequence (SEQ ID NO: 18).

\[
\begin{align*}
1 & \text{GCGAGCAGCG AGTGACAGGA CGTCTGAGCA GGGCGGCGGC} \\
41 & \text{TACGAGCTCT GCGGGCCGCC GGGGTGATAC GATGCGGGCG} \\
81 & \text{CTGGAGACC ACCAGCCGAG TGAGGGCCGA CAGCCGGCG} \\
121 & \text{CCGAGGCCGC GGGCGGAGA CGCAGCGCGG ACGCCGGCG} \\
161 & \text{TCCGCGCAAG AGTCGAGGCC CCGCGGGCGG GTTGAATTTG} \\
201 & \text{TATAGGTGTT GCAACAATT CGACCGAGTT GATCAAAATG}
\end{align*}
\]
241 ATTCTCAACT CTTCCTAGAA AGATGGTATT AAAAGAATCC
281 AAGATGATTG TCCCAAGCTT GGAAGGCATA ATTACATATT
321 TGTCATGATT CCTACTTTAT ACAGTATCAT CTGTGTTGTC
361 GGAATAATTTG GAAACAGCTT GGTGGTATGA GTGATTTACT
401 TTTATATTCG TCTGATGACTA GTGGGTAACT TAAAATATTCC
441 ATTCTCAACT CTTCCTAGAA AGATGGTATT AAAAGAATCC
481 CTGCAATCTG TCCCAAGCTT GGAAGGCATA ATTACATATT
521 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
561 CAGTTTCAAC CTGTCACGCTA GTGGGTAACT TAAAATATTCC
601 ATTCTCAACT CTTCCTAGAA AGATGGTATT AAAAGAATCC
641 AGTCCCCGCT TCGACGCACA ATGCTTTGTG TTTAATTCTG
681 TGTCATGATT CCTACTTTAT ACAGTATCAT CTGTGTTGTC
721 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
761 CCAATATTAC ATGTTGTGCT TTTCAATTATG AGTCCCAAAA
801 TTTCAACCCT GCATCAAGCTT GGTGGTAACT TAAAATATTCC
841 CTGTGATAAT GCATCAAGCTT GGTGGTAACT TAAAATATTCC
881 TTTCAACCCT GCATCAAGCTT GGTGGTAACT TAAAATATTCC
921 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
961 CCAATATTAC ATGTTGTGCT TTTCAATTATG AGTCCCAAAA
1001 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1041 AGTCCCCGCT TCGACGCACA ATGCTTTGTG TTTAATTCTG
1081 ATGCTTTTGT TTTGGAAGGCC CTGGCTGACT TTTTGTGGTG
1121 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1161 CCAATATTAC ATGTTGTGCT TTTCAATTATG AGTCCCAAAA
1201 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1241 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1281 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1321 AGTCCCCGCT TCGACGCACA ATGCTTTGTG TTTAATTCTG
1361 CCAATATTAC ATGTTGTGCT TTTCAATTATG AGTCCCAAAA
1401 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1441 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1481 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1521 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1561 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1601 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1641 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1681 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1721 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1761 CCGTGGCAA TTACCGACAT AAGATGACTA CATCCAAGTA
1801 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1841 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1881 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1921 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
1961 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
2001 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
2041 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
2081 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
2121 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG
2161 ATGTGACATT CTGGCTGACT TTTTGTGGTG TTTTGTGGTG

In addition to angiotensin, angiotensin II receptors and ACE, renin has been shown to be a potent vasoconstrictor that can result in high blood pressure. Renin converts angiotensinogen to angiotensin I which can result in vasoconstriction due to the down-stream effects (angiotensin-I conversion to angiotensin II through ACE). One example of a functional and common renin polymorphism (Vangjeli et al, Circulation Cardiovascular genetics 3:53-59 (2010)) can influence the blood pressure response to vasodilator therapy. This renin polymorphism is present in rsl2750834. The nucleotide sequence surrounding the renin rsl2750834 single nucleotide polymorphism is shown below, where the underlined A/G in the sequence (SEQ ID NO: 19) is the SNP. AGAACACCAAAGCAAGGCT TAATCTG [A/G]GGGCAC TACAGAGACTGCT TTAA A.

The complementary sequence of SEQ ID NO: 19 is the following sequence (SEQ ID NO:20).

TTAAAGCAGTCTCTGTAAGTGCCC [C/T]CAGAT TAAGGCTGCT TTGTTGT TC T.

Note that the cytosine to thymine substitution is a guanine to adenine substitution in the opposite strand.

The rsl2750834 SNP contains a cytosine to thymine substitution, or a guanine to adenine substitution depending upon the DNA strand, at about nucleotide position 5312 upstream of the renin start site. The cytosine (guanine) variant of renin has been shown to correlate with greater reduction in blood pressure upon administration of angiotensin II receptor blockers such as valsartan.

Sodium / Diuretic Regulation of Blood Pressure

The kidneys are the center of long-term blood pressure regulation. Alterations in Na\(^+\) reabsorption in the kidneys result in alterations in fluid retention, which leads to increases or decreases in blood plasma volume as well as to changes in the pressure against the vessels. There are several proteins that are important in renal Na\(^+\) handling and in the response to diuretic therapy including the epithelial Na\(^+\) channels, alpha-adducin, the Na\(^+\)Cl\(^-\) co-transporter, and lysine deficient protein kinase- 1 (WNK).

The epithelial sodium (Na\(^+\)) channel is responsible for Na\(^+\) reabsorption on the apical portion of epithelial cells in the kidneys. The Na\(^+\) channel is made
up of three different subunits: the alpha, beta, and gamma. The alpha subunit of the epithelial Na\(^+\) channel is highly functional and removal of this subunit abolishes channel activity in cell and animal models. The gamma subunit is also extremely important in channel function. Functional gamma genetic variants result in pseudohypoaldosteronism type-I and Liddle's syndrome, two severe genetic diseases resulting in salt wasting and high salt conservation (salt sensitivity), respectively. Adducin is made up of an alpha, beta, and gamma subunit. The alpha subunit increases sodium (Na\(^+\)) reabsorption in the kidneys through the activity of Na\(^+\)K\(^+\) ATPase (which moves Na\(^+\) and potassium into and out of cells). The sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter is important in regulating Na\(^+\) and Cl\(^-\) movement between the kidney and the rest of the body. Active Na\(^+\)-Cl\(^-\) transport results in Na\(^+\) reabsorption and can, therefore, result in higher blood pressure. The WNK1 protein is a key regulator of long-term Na\(^+\) and chloride Cl\(^-\) reabsorption in the kidneys. WNK1 regulates the activity of Na\(^+\)-Cl\(^-\) co-transporters. If a patient has a more active WNK1 genotype, they likely have greater Na\(^+\) and Cl\(^-\) reabsorption in the kidneys which can increase blood volume and, therefore, pressure on the vessels.

A functional and common polymorphism of the gene that encodes the epithelial Na\(^+\) channel (SCNN1A) has been identified, where the polymorphism is an alanine to threonine substitution at about position 663-722. A cDNA sequence for the human SCNN1A gene is available from the NCBI database as accession number NM_001159576.1 (GL227430288). This sequence is provided below as SEQ ID NO:21.

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1  AAACAGAAGG CAGATAGAGA GGGAGTGAGA GCCAGAGCT
41 GAGACACAGA TCCCTGAGGA AGAAGACCAA AGGAAGGGGG
81 CAGAGACAGA AAGGGAGGTT TAGGACAAA ACTCGAAAGG
121 TGGCCCTATC AGGGAAGCAG AGGAGAGCC GTTCTAGGGA
161 AGCCCAGCTC CGGCACCTTT GCAGCCAACCT CCCAGGCTC
201 TGCTGCTGCTC AGAAAGGTG GAGAGGGGAG GAGAGGTTG
241 GAGAATGTGG GGGCAGGGGTA GGAGATGGG ATGGCCAGGG
281 GCAGCCTCAC TCGGGTTCAA GGGGTGATGG GAGAGGGCAC
321 TCAAGCCCAA GAGCTCAGCC TTGACCTGTA CACCCCTTCT
361 CCCCATACTC CTCGGGGGCT CATGAAGGGG AAACAGCTGG
401 AGGAAGCAGGA CCTGAGGCTT CTGAGGAGCC TACAGGGTCT
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**Total lines**: 50
The rs2228576 single nucleotide polymorphism (SNP) is present in the SCNNIA gene, where the variable nucleotide is at about position 2428 in SEQ ID NO:21 (underlined), which can be adenine in some individuals and guanine in others. The rs2228576 sequence (SEQ ID NO:22) is shown below, where the underlined A/G is the SNP.

```
GGGCTCTGCAGGGGCCAGTTCCTCC [A/G] CCTGTCCTCTGGGGGGGCCCTGAG
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The human the epithelial Na+ channel encoded by the SCNNIA cDNA with SEQ ID NO:21 has the following sequence (SEQ ID NO:23).

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81  KGKREEQGL GPEPAAPQPQ TAEDELIEF HRSYRELFEF
121 FCNNTTHGA IRLVCSQHNR MKTAFWAVLW LCTFGMMYWP
161 FGLLFGEYFS VPVSNINLNL SDKLVFPAVT ICTLNPYRP
201 EIKEELEELD RITEQTLFDL YKYSSTTLV AGSRSRRDLR
241 GTLPHPQLRQL RPVPPPHGAR RARSVASSLR DNNPQVDWKD
281 WK1GFQLNCNQ NKSDFCYQTY SSVDVAVREW YRFHYINILS
321 RLPEFLPSLE EDTLGNFIFA CRFNQVSNQNY NSHYSHHHPM
361 YGNCTTTFNDD NNSNLMSSS PGINGNLSLM LRAEQNDFIP
401 LLSTVTGARV MVHGQDEPAF MDDGGFNLRP GVETSISMRK
441 ETLDRLGQY GDIEKNGSVDV PVENLYPSKY TQQVCICSHC
481 QESMKECGC AYIFYPRPQUN VEYCYRKHS SWGYYKLQ
521 VDFSSSDLGC FTCKRKPSCS TSYSLSAGYS RYPSVTSQEW
561 VFQMLSRQNN YTVNKNRNGV AKVNIVFKEI NYKTNSESPE
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Another cDNA sequence for the human SCN1A gene with the same SNP is available from the NCBI database as accession number NM_001038.5 (GL227430285). This sequence is provided below as SEQ ID NO:24.

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1  CTTGCCTGTC TGCGTCTAAA GCCCCTGCCC AGAGTCCGCC
10 41 TTCTCAGGTC CAGTACTCCC AGTTCACCTG CCCTCGGGAG
15 81 CCCTCCTTCC TTCGGAAAAC TCCCGGCTCT GACTCCTCCT
20 121 CAGCCCCTCC CCCCCGCCCTG CTCACCTTTA ATTGAGATGC
25 161 TAATGAGATT CCTGTCGCTT CCATCCCTGG CCGGGCCAGCG
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35 241 CAAGCTGGAG GAGCAGGACC CTAGACCTCT GCAGCCCATA
40 281 CCAGGTCTCA TGGAGGGGAA CAAGCTGGAG GAGCAGGACT
45 321 CTAGCCCTCC ACAGTCCACT CCAGGGCTCA TGAAGGGGAA
50 361 CAACACCACC ATCCACGGCG CCATCCGCCT GGTGTGCTCC
55 401 CAGCACAACC GCATGAAGAC GGCCTTCTGG GCAGTGCTGT
60 441 TCCACCGCTC CTACCGAGAG CTCTTCGAGT TCTTCTGCAA
65 481 CAACACCACC ATCCACGGCG CCATCCGCCT GGTGTGCTCC
70 521 CAGCACAACC GCATGAAGAC GGCCTTCTGG GCAGTGCTGT
75 561 GGCTCTGCAC CTTTGGCATG ATGTACTGGC AATTCGGCCT
80 601 GCTTTTCGGA GAGTACTTCA GCTACCCCGT CAGCCTCAAC
85 641 ATCAACCTCA ACTCGGACAA GCTGTTCCTC CCCGCAGTGA
90 681 CCATCTCGTCT ATACCTTGAA TGGAGGGGAA CAAGCTGGAG
95 721 GAACCTGCCT TTATGGATGA TGGTGGCTTT AACTTGCGGC
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105 801 TGGCGCGGCTC CGGAGCGCGT CGCGACCTGC GGGGGACTCT
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115 881 CAGGGGGCCC TGGAGGGGAA CAGCTCCTTC ACCTCCTTCG
120 921 GGGCAGCGTA CACTCCTGCT CCAAGTGGTA AACTTGCGGC
125 961 CCGGTTCGCA CGTTCGGAACT GAAACAAATC GGACTGCTTC
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135 1041 GGTACCCTTT CCACTACATC AACATCCTGT CGGAGGCTGCC
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145 1121 TTCACTCCTGC TCTGGAGTGC ATGTCCCTTG TCAGCTCGTC
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The human epithelial Na⁺ channel encoded by the SCNN1A cDNA with sequence (SEQ ID NO:25).
Note that the underlined threonine at position 722 of the SEQ ID NO:23 SCNNIA protein, and the underlined threonine at position 663 of the SEQ ID NO:25 SCNNIA protein, is threonine because some individuals have nucleotide sequence SEQ ID NO:22, where the variable nucleotide is adenine. However, position 722 of SEQ ID NO:23 and position 663 of SEQ ID NO:25 can be alanine in some individuals because those individuals have guanine as the variable nucleotide in sequence SEQ ID NO:22.

Patients with the threonine substitution in SCNNIA (adenine in rs2228576) have more functional Na\(^+\) channels and consequently higher activity higher voltage currents across the cells. Hence, patients with such a threonine at the variable site in SCNNIA are more susceptible to hypertension than SCNNIA proteins with alanine at that position. Patients with the threonine substitution in SCNNIA can benefit from administration of amiloride.

Common and functional genetic variation of alpha adducin at amino acid 460 has also been identified where some individuals have glycine and others have tryptophan. A cDNA sequence for the human alpha adducin gene (ADD1) is available from the NCBI database as accession number NM_001119.4 (GL346644753). This ADD1 sequence is provided below as SEQ ID NO:26.

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81 ACGGGGCAGG AGCCGGAGCC GAGCGGACGC GGGGTGGCC
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161 CTTCTGAGGA ACCTAGAAG ATTTGACAAT GAATGGTGAT
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241 CCCCTCACAAG GAGAGGTAC TGGAGGTAC GAGATGAGAA
281 CAAACCAGAG CCTTACATGA AGAGGAACAT GGCACCAGAC
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601 ATGACAAAGG AGAGAAGTGA TTACGGTGTA AATTGGCAGC
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801 GATATAGTAG ATCGTGGGAG CACTAATCGT GGAGTGAATC
841 AGGCCGCGTT CACCTTACAC TCTGCAATTT ATGCTGACAG
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15 961 TCTCCGGGA GCGTCCTTCC TGGTGAGAAG TGGTCTATCA
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45 2361 CGTCTCTTTC TGAAAGAGGA CAAAGAAGAG AGTGACTCTC
2401 GAAAGCCTGG CGCTAACCAG TCTCGTCCGG GAGCGACCCT
| 2441 | GGCTCTGCCA | GCGTCCCCGG | CCACGTCTGT | GCTCTGTCCT |
| 2481 | TGTTGAAAGG | AATGCAAAGG | AGCCAAAGCC | TCCGGCCTAGA |
| 2521 | GGCTCCCCCTA | GTGACCAAGC | CGGCAGCCAG | CAGGGCCCTAG |
| 2561 | CCAAGTGGTGT | GCTACGACGC | CCCACCCACCC | CCTGCCCTCTT |
| 2601 | GTCTCTCAAG | AGCTCTCAAG | TCTGGGGGGT | ACGTACCTCTC |
| 2641 | CACAGGGGGA | GAGGCCACTA | AGTCAATGGT | CGTGGCCTAGA |
| 2681 | GGTACGGAGG | GCTTCTGCAAG | TCTGGCTGCTG | ACGTACCCCTC |
| 2721 | CCTAGGCCCT | ATCTTCTCT | CGTCCCTCTC | TTTGGTGAAG |
| 2761 | TCAACACATT | GTCTCTGACT | TTTGCTTCCC | TCTTCAACCTA |
| 2801 | AAGTTCAGGT | TGACCGAGCT | AGTCTGAGTC | CCCACCCCTAG |
| 2841 | CATTCTCTCT | GCTCAGTGATC | CTCACTTAAA | TCTATATACA |
| 2881 | AAGGGCTTGG | CCCGCTTTCA | CAATGGCTAT | CCCACCCCTAG |
| 2921 | GCCCTGAAGG | GGCAGCTCTG | GGCAGAGTTT | TGGAGGCCGA |
| 2961 | ACCCGGCTGG | CGACTGGGCC | TCGTGCAACC | CAAAGTGATG |
| 3001 | CTATGACAGT | TGTACGACGC | CCCACCCCTAG | AGAATCAGAG |
| 3041 | TTCTTAGGCT | GAGGCAGCTT | TCTGAGGGCC | TGCTGGTGTG |
| 3081 | CCTGCTAGAG | GGAAGGCTTG | ATCAAGAGTA | ATCGGAGGTC |
| 3121 | TCTGAGAATT | TTTGGTCTAT | TTAGCTTACA | TGGCAGCTAGA |
| 3161 | TTCTTTGACT | GTGAAGCTCT | AGTGGCTGCTG | TTTGGTCTTTC |
| 3201 | TTCTTTTACG | ACCGGCAAGT | AGACCTCATT | TCACATATT |
| 3241 | GGGGGAGGCT | TACAGGGCTT | TCTAGTCTGG | TCTCTGAGGC |
| 3281 | AGAAAGATGC | CCTAGTCTTG | ATCAAGAGTA | ATCGGAGGTC |
| 3321 | GAGGGTACGG | CGGGGCTCTG | TCACTTCAAT | TGCTGAGGCT |
| 3361 | GGAGCTGGAA | CGTTGCTCCT | CTGGCAGATG | GTCTGGTGTG |
| 3401 | TTACCTTCCT | CCAAGAGGCT | GGAAGGGCCT | TCAGAGGCTC |
| 3441 | TGGACGGAGA | CCGGGGCTTT | ACCAGTTTGA | GGGGCTCCC |
| 3481 | GTACGGAGCT | GACTAGTATG | AGGAGAGTTT | GTGAGGGGCC |
| 3521 | AAGACGGCAA | TGGTACAGTAT | GTGCCAGATG | TGGGCTCAGA |
| 3561 | ATCTCTGGCA | TGATGGGCCG | AGCCAATCTG | GATCCAATGT |
| 3601 | ATCGGACAGC | CGAGGAGGA | CCGAGGCTCT | GCAGAGGCCT |
| 3641 | GACCGGGGCT | TCACTTATTC | TCTAGGACGG | AGGGCTGCTT |
| 3681 | AGCGGGCAA | GCGTCTGCTC | TCTTCTCTGT | TGCGTTTTGT |
| 3721 | TGGCAGGAGA | GAATTAGAGAAG | TCTTTACCCC | TGGAGCTTTC |
| 3761 | GTCCTTACCC | TCTTTTACCC | CACATAGTCA | ATGACTTTT |
| 3801 | ATTTTGTATT | TTGTAGTTTT | ATCCCTTGTGA | TTTACATAG |
| 3841 | AATATATAGT | GCAATAGTGC | AGCCAGCCTT | GGGGAGGAGA |
| 3881 | GTCCTTAGAT | TCTTTTTCCT | AGTGAACAT | GTTATTTTCT |
| 3921 | CAAATAAATG | TCTTTATACG | AGTTCCCTGT | CTAAAAAAAA |
| 3961 | AAAAAAAAA | | | |

The rs4961 single nucleotide polymorphism (SNP) is present in the ADD1 gene, where the variable nucleotide is at about position 1566 in SEQ ID NO:26 (underlined), which can be guanine in some individuals and thymine in others. The rs4961 sequence (SEQ ID NO:27) is shown below, where the underlined G/T is the SNP.

```
CCGGGGCGACGAAGCTTCCGAGGAA [G/T] GGCAGAATGGGAAGCAGCTTCCCAAGG
```
The human alpha adducin encoded by the ADD1 cDNA with SEQ ID NO:26 has the following sequence (SEQ ID NO:28).

1 MNGDSRAAVV TSPPPTTAPH KERYFDRVDE NNPYPELRENN
41 MAPDLRDQDFN MMEQKKRVSM ILQSPAFCEE LESMIQEQQFKN
81 KGKNFTGLLA LQQIADFMNT NVPNVPAPAP QGGMMAALNMS
121 LGMVTQPVNLQ RGDSSIAAYDK GEKLLRCLKA AFYRLADLF
161 WSQLIYNHIT TRVNSEQEHF LIVPFGLLLYS EVTASSLVIK
201 NLQGDIVDRG STNGLVNCAG FTLHSAYAA RPVDKCVCVHI
241 HTPGAAVSA MKCGLLPISP EALSLGEVAY HDHYGILVED
281 EEKVLIQKLNL GPKSVKLILR NHGLVSVGES VEEAFYIYHN
321 LVVACEIQVR TLSAGGPDPN LVLLNPEKYK AKSRSPPGSPV
361 GEGTGSPPKW QIGEQEFEAL MRMLDNLGYR TGYPYPLPAL
401 REKSKKYSDDV EFPASVTGYS FASDGDSGTC SPLRHSFQKQ
441 QREKRWNLNS GRGDEASEEQ QNGSSPDKST KWTKEDGHRT
481 STSAPVNLFV PLNTPKVEQ EMRNKIREQN LQDIKTAGPQ
521 SQVLCGVVMDD SLVQGGELVT ASKAI IEKEY QPHIVSTTG
561 PNPFTTLDTR ELEEVRREVE RKQKGSEENL DEAREQKEKS
601 PDDQPAVPHP PSPSTIKLEE DLVPEPTTGD DSDAATFKPT
641 LDPDLSPDEPS EALGPMLEKK EEEAHRRPSP TEAPTEASEP
681 PAPDPAPVAEE EAAPSAVEEG AAADPGSDGS PGKPSKSKK
721 KFRTFSFLKK SKKKS

Note that the underlined glycine at position 460 of the SEQ ID NO:28 alpha adducin protein is glycine because some individuals have nucleotide sequence SEQ ID NO:26, where the variable nucleotide at position 1566 is guanine. However, position 460 of SEQ ID NO:28 can be tryptophan in some individuals because those individuals have thymine as the variable nucleotide at position 1566 in sequence SEQ ID NO:28.

Individuals with the tryptophan variant of alpha adducin are more likely to be salt sensitive, more likely to have hypertension and have a greater response to diuretics.

Genetic variation of the sodium (Na⁺) chloride (Cl⁻) co-transporter (SLC12A3) also has blood pressure consequences. A cDNA sequence for the sodium (Na⁺) chloride (Cl⁻) co-transporter (SLC12A3) is available from the NCBI database as accession number NM_000339.2 (GL186910314). This SLC12A3 cDNA sequence is provided below as SEQ ID NO:29.

1 CTGCCCCCTC CTCGGACACC CAGGGCGACA TGGCAGAACT
41 GCCCAACAAC AGAGCAGGCTG GGAGCCGAC TTTGTCGACG
81 GGGCGCTTCA CCATCAGCAC AGTGTGAGC AGTGTAGGAC
121 CCTCCTCACC AGCGCCCTAT GACAGCAAGCC ACCCCAGCG
161 CCTGACCCAC AGAGCAGCCT TCTGATTCG CACCTTTG
201 TACACACAGA TCAGTTGGTG GCCCCATAT AGCAGTATG
241 CCAACACGAC CCAAGCTTGG CAGCCCCGGA AGGTCGGG
281 CACACTGCGT GACCTGACACT CTTCTCTCAA GCAGGAAGGC
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The rs1529927 single nucleotide polymorphism (SNP) is present in the SLC12A3 gene, where the variable nucleotide is at about position 820 in SEQ ID NO:29 (underlined), which can be guanine in some individuals and cytosine in others. The rs1529927 sequence (SEQ ID NO:30) is shown below, where the underlined C/G is the SNP.

```
CCCATTAACGACATCCGCATCATTG [C/G] CGTGGTCTCGGTCACTGTGCTGCT
G.
```

The human the sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter encoded by the SLC12A3 cDNA with SEQ ID NO:29 has the following sequence (SEQ ID NO:31).

```
1  MAELPTTETP GDATLCSGRF TISTLLSSDE PSPPAAYDSS
41 HPShLTHSSST FCMRTFGYNT IDVVPTEHY ANSTQPGEPR
81 KVRPTLADLH SLFKQEGRLH HALAFDSRPS HEMTDGLVEG
121 EAGTSSEKNP EEPVRFGWVK GVMIRCMLNI WGVILYLRLP
161 WITAQAGIVL TWIIILLSVT VISITGLSIS AISTNGKVKS
37
```
| 201 | GGTYFLI SRS | LGPELGGSIG | LIFAFANAVG | VAMHTVGFAE |
| 241 | TVRDLQVYG | APIVDPINDI | RIIGVSVVT | LIAI SLAGME |
| 281 | WESKAQVLFF | LVIMVSFANY | LVGLTIPPSE | DKASKGFSSY |
| 321 | RADIFVQNLV | PDRGPDGTF | FGMSIFFPS | ATGILAGANI |
| 5 | SGLDKDPFIA | IPKGTLMMAIF | WTTSYLAIS | ATIGSCVVRD |
| 401 | ASGVLNNDTVT | PGWGACEGLA | CSYGWNNFTEC | TQQHSCHYGL |
| 441 | INYYQTMSMV | SGFAPLITAG | IFGATSSLAL | ACLVSAAKVF |
| 481 | QCLCEDQLYP | LIGFGKGYG | KNKEPVRGYL | LAYAIAVAFI |
| 521 | IIAELNTIAP | IISNFFLCY | ALSINFSCFHA | SITNSPGWWRP |
| 561 | SFQYNYNKWA | LFGAIIISVVI | MFLLTWAAAL | IAIGVVLPLL |
| 601 | LYVI YKKPEV | NWGSSVQAGS | YNALSYSVG | LNEVEDHIKN |
| 641 | YRPQLVLTG | PPNFPRALVD | FVGTFTRLNS | LMICGHVLLG |
| 681 | PHKQRMPELQ | LIANGHTKWL | NKRKIKAFYS | DVIAEIDLRRG |
| 721 | VQILMQAAGL | GRMKPNILVV | GFKKNWQSAH | PATVEDYIGI |
| 761 | LHDADFDFNYG | VCVMRMREG | NVSKMMAHI | NVPFDPAEDG |
| 801 | KEASARGARP | SVSGALDPKA | LVKEQATTI | FQSEQQKKT |
| 841 | DIYWLFDGGG | LTTLLI PYLLE | RKRRWSKCKI | RVFVGQGINR |
| 881 | MDQERKAI LS | LSFKRFLGFI | EVHILPDIQ | NPRABXHTKF |
| 921 | EDMIAPFRLN | DGFKDEATVM | EMRRDCEWIKI | SDEEITKNVR |
| 961 | KSLRQVRLNE | IVLDYSRDAA | LIVITLPIGR | KGKCPSSLYM |
| 1001 | AWLETLSQDL | RPPVILIRGN | QENVLTFYCQ |

Note that the underlined glycine at position 264 of the SEQ ID NO:3 indicates that the sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter protein is glycine because some individuals have nucleotide sequence SEQ ID NO:29, where the variable nucleotide at position 820 is guanine. However, position 264 of SEQ ID NO:29 can be alanine in some individuals because those individuals have cytosine as the variable nucleotide at position 820 in sequence SEQ ID NO:29.

Patients with the alanine variant of SLC12A3 (encoded by the rs1529927 site (SEQ ID NO:30)) exhibit a stronger diuretic effect to loop diuretics and demonstrate more excretion of CI and K\(^+\) in response to therapy. Hence, subject with alanine or guanine at the rs1529927 site are more response to diuretics.

The WNK1 gene has functional and common polymorphisms that affect how a subject's blood pressure responds to drugs. Several cDNA sequences for the WNT1 gene are available from the NCBI database.

The rs2107614 single nucleotide polymorphism (SNP) is present in an intron of the WNK1 gene, where the variable nucleotide can be thymine in some individuals and cytosine in others. The rs2107614 sequence (SEQ ID NO:33) is shown below, where the underlined C/T is the SNP.

\[
\text{CACTTCCCT CCAAAAAAAG AAAAC [C/T] CCATTCCCCCTCAACTCTTCCAGT T.}
\]
Another SNP, rsl 159744, is present an intron of the WNK1 gene, where the variable nucleotide can be guanine in some individuals and cytosine in others. The rsl 159744 sequence (SEQ ID NO:34) is shown below, where the underlined C/G is the SNP.

```
AATGT TAACAGTAGAAAAT TTA [C/G] CTCAACAAATAGAGAATATCAGTA A.
```

Patients with the cytosine variant of WNK1 at SNP positions rsl 159744 and rs2107614 exhibit greater blood pressure reductions in response to loop diuretic therapy when compared to patients with the guanine or thymine variants at these two sites, respectively (Turner et al, *Hypertension* 46:758-765 (2005)).

**Therapy**

The methods, reagents, devices, and kits described herein can be used for determining whether a subject may benefit from treatment with a blood pressure medication, and which medication can be more effective for treating high blood pressure. For example, the methods described herein can be employed for determining whether a subject should be treated with a diuretic, an angiotensin converting enzyme (ACE) inhibitor, or a beta-blocker. Such determination is performed by identifying or detecting whether the subject has a genetic variant or single nucleotide polymorphism in his or her ADRB1, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na⁺ channels (such as SCNN1A), adducin, sodium (Na⁺) chloride (Cl⁻) co-transporters (such as SLC12A3), and/or WNK1 polypeptides or nucleic acids. If testing of the subject's tissue sample shows that the subject has a genetic variant or single nucleotide polymorphism in his or her ADRB1, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na⁺ channels (such as SCNN1A), adducin, sodium (Na⁺) chloride (Cl⁻) co-transporters (such as SLC12A3), and/or WNK1 polypeptides or nucleic acids, a suitable therapeutic regimen can be prescribed for the subject.

A diuretic promotes the production or urine. Diuretics are sometimes grouped into three categories: thiazides, loop, and potassium-sparing diuretics. Thiazide diuretics include chlorothiazide, hydrochlorothiazide, indapamide, metolazone, and chlorthalidone. Loop diuretics include furosemide, bumetanide,
ethacrynic acid, and torsemide. Examples of potassium-sparing diuretics include amiloride, eplerenone, spironolactone, and triamterene.

Examples of diuretics that can be employed also include furosemide, thiazides, carbonic anhydrase inhibitors, potassium-sparing diuretics (e.g., aldosterone antagonists, spironolactone, eplerenone, potassium canrenone, amiloride, triamterene, aldactone, and combinations thereof), calcium-sparing diuretics. For example, the diuretic can be acetazolamide, amiloride, bumetanide, chlorothalidone, chlorothiazide, ethacrynic acid, furosemide, glycerin, hydrochlorothiazide, hydroflumethiazide, indapamide, isosorbide, mannitol, methazolamide, methylchlorthiazide, metolazone, dichlorphenamidine, spironolactone, torsemide, triamterene, urea, and combinations thereof.

The angiotensin converting enzyme inhibitor can be selected from enalapril, lisinopril, captopril, alacipril, benazapril, cilazapril, delapril, fosinopril, perindopril, quinapril, ramipril, moveltipril, spirapril, ceronapril, and combinations thereof.

The angiotensin II receptor antagonists can, for example, be losartan, valsartan, candesartan, irbesartan, olmesartan, or any combination thereof.

The renin inhibitors can be urea derivatives of di- and tri-peptides (See U.S. Pat. No. 5,116,835), amino acids and derivatives (U.S. Pat. Nos. 5,095,119 and 5,104,869), amino acid chains linked by non-peptidic bonds (U.S. Pat. No. 5,116,937), di- and tri-peptide derivatives (U.S. Pat. No. 5,106,835), peptidyl amino diols (U.S. Pat. Nos. 5,063,208 and 4,845,079) and peptidyl beta-aminoacyl aminodiol carbamates (U.S. Pat. No. 5,089,471); also, a variety of other peptide analogs as disclosed in the following U.S. Pat. Nos. 5,071,837; 5,064,965; 5,063,207; 5,036,054; 5,036,053; 5,034,512 and 4,894,437, and small molecule renin inhibitors (including diol sulfonamides and sulfinyls (U.S. Pat. No. 5,098,924), N-morpholino derivatives (U.S. Pat. No. 5,055,466), N-
heterocyclic alcohols (U.S. Pat. No. 4,885,292) and pyrolimidazolones (U.S. Pat. No. 5,075,451); also, pepstatin derivatives (U.S. Pat. No. 4,980,283) and fluoro- and chloro-derivatives of statone-containing peptides (U.S. Pat. No. 5,066,643). enalkrein, RO 42-5892, A 65317, CP 80794, ES1005, ES 8891, SQ 34017, aliskiren ((2S,4S,5S,7S)—N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-oetanarriid hemifumarate) SPP600, SPP630 and SPP635), or any combination thereof.

Other therapeutic agents can also be administered including endothelin receptors antagonists, vasodilators, calcium channel blockers (e.g., amlodipine, nifedipine, verapamil, diltiazem, gallopamil, nifedipine, nimodipins, nicardipine), potassium channel activators (e.g., nicorandil, pinacidil, cromakalim, minoxidil, aprilkalim, loprazolam), diuretics (e.g., hydrochlorothiazide), sympatholitics, beta-adrenergic blocking drugs (e.g., propranolol, atenolol, bisoprolol, carvedilol, metoprolol, or metoprolol tartrate), alpha adrenergic blocking drugs (e.g., doxazocin, prazocin or alpha methyldopa) central alpha adrenergic agonists, peripheral vasodilators (e.g. hydralazine), lipid lowering agents (e.g., simvastatin, lovastatin, ezetamibe, atorvastatin, pravastatin), metabolic altering agents including insulin sensitizing agents and related compounds (e.g., muraglitazar, glipizide, metformin, rosiglitazone) or with other drugs beneficial for the prevention or the treatment of disease including nitroprusside and diazoxide.

The therapeutic protocol can generally be conducted as follows. An assay of all sixteen genotypes (polymorphic sites) can be performed. The therapeutic decision tree of the results can be as follows.

To ascertain whether a diuretic should be administered to a subject, the following can be performed.

- If the subject is (a) homozygous for cytosine at the rs1529927 (SEQ ID NO:30) variable site (expressing alanine at position 264 of the SLC12A3 gene product); (b) homozygous for adenine at the rs2228576 (SEQ ID NO:22) variable site (expressing threonine at about 663 or 722 of the SCN1A protein); and/or (c) homozygous for thymine at the rs4961 (SEQ ID NO:27) variable site (expressing tryptophan at about position 460 of the adducin protein) then the patient should initially start with a diuretic as the first line of therapy. If the patient is heterozygous at these
sites, then genetic variation within the polymorphic sites relating to vasodilator and beta-blocker drug class responses should initially be considered.

- If the patient does not carry homozygous variants that are known to be functionally important within the vasodilator and beta-blocker classes, but are heterozygous at rs1529927, rs2228576, and rs4961, then diuretic therapy should initially be considered as first-line therapy.

- If the subject is homozygous for cytosine at the WNK1 rs1 159744 (SEQ ID NO:34) variable site and also homozygous for cytosine at the WNK1 rs2107614 (SEQ ID NO:33) variable site then the patient should start with a loop diuretic as first-line of therapy.

- If the patient does not carry homozygous variants within the vasodilator and beta-blocker classes that are known to be functionally important, but are heterozygous at rs1529927, rs2228576, and rs4961, then loop diuretic therapy should initially be considered as first-line therapeutic agent.

To ascertain whether a vasodilator should be administered to a subject the following can be performed.

- If the subject is homozygous for cytosine at the rs5 186 (SEQ ID NO:16) variable site of AGT1R, and the subject is homozygous cytosine at the rs12750834 (SEQ ID NO:20) variable site of renin, then the patient should use an angiotensin II (All) receptor blocker as a first line of therapy.

- If the patient is heterozygous for cytosine at the rs5 186 and rs12750834 variable sites, but does not present with other important functional genotypes within the diuretic and beta-blockade classes, then the patient should also use an angiotensin II receptor blocker as a first line of therapy.

- If the patient is homozygous for cytosine at the rs699 (SEQ ID NO:14) variable site of AGT, or for the deletion at the rsl 799752 (SEQ ID NO:12; SEQ ID NO:35) of ACE, then the patient will likely benefit most from an angiotensin-converting enzyme (ACE) inhibitor.

- Patients who are homozygous for cytosine at the rs699 (SEQ ID NO:14) will likely benefit most from **BOTH** ACE inhibition and angiotensin II (All) receptor blockade.
Patients who are heterozygous for the deletion at the rs1799752 (SEQ ID NO: 12; SEQ ID NO:35) and heterozygous for cytosine at the rs699 (SEQ ID NO: 14) variable site should be administered other drug classes (e.g., diuretic initially followed by beta-blockade). Although homozygosity at other sites has a greater impact on hypertension than heterozygosity at rs1799752 and rs699, this is generally true only if the patient has combined homozygosity at sites indicating that drug classes other than vasodilators should be administered.

To ascertain whether a beta-blocker should be administered to a subject the following can be performed.

• Patients homozygous for adenine at the rs3892097 (SEQ ID NO:10) variable site of the CYP2D6 gene should initially consider the use of atenolol and carevolol as therapy. This is PARTICULARLY important if the patient is homozygous for cytosine at the rs1801253 (SEQ ID NO:3) variable site (and expresses arginine at position 389 of the βAR polypeptide), or if the patient is homozygous for adenine at the rs1801252 variable position (and expresses serine at position 49 of the PiAR polypeptide).

• The rs1042713 (SEQ ID NO:6) and rs1042714 (SEQ ID NO:7) variable sites are less important of the other polymorphism sites within the beta-blocker class of drugs and generally indicate patients who will likely respond to non-selective beta-blockade. Thus, subjects who are homozygous for guanine at the rs1042713 variable site (and express glycine at about position 16 of the ADRB2 gene product) as well as subjects who are homozygous for guanine at the rs1042714 position (and express glutamic acid at β2AR position 27) are the most responsive to beta-blocker drugs.

• If subjects are non-homozygous for polymorphisms in the beta-blockade class of variants, but are homozygous for cytosine at the rs1801253 (SEQ ID NO:3) variable site (and expresses arginine at position 389 of the β1AR polypeptide), or if subjects are homozygous for adenine at the rs1801252 variable position (and expresses serine at position 49 of the β1AR polypeptide), the beta-blockade class should be considered a
possible line of therapy if they do not carry functional mutations within
the diuretic and vasodilator classes of drugs.

Polymorphism Detection

The polymorphism present in genes such as ADRB1, ADRB2,
cytochrome P450 2D6 (CYP2D6), angiotensin converting enzyme (ACE),
angiotensinogen, angiotensin receptors, renin, Na⁺ channels (such as SCNNI A),
adducin, sodium (Na⁺) chloride (Cl⁻) co-transporters (such as SLC12A3), and/or
WNK1 can be detected by any available procedure. For example, samples of
cDNA, genomic DNA, and/or mRNA can be obtained from a subject and the
sequences of polymorphic or variant sites can be evaluated by procedures such
as nucleic acid amplification (e.g., PCR), reverse transcription, insertion/deletion
analysis, primer extension, probe hybridization, SNP analysis, sequencing,
restriction fragment length polymorphism, Matrix-Assisted Laser
Desorption/Ionization Time-Of-Flight mass spectrometry (MALDI-TOF MS),
Sequenom MassArray genotyping, Sanger sequencing, polyacrylamide gel
electrophoresis, agarose gel electrophoresis, probe array hybridization analysis,
and combinations thereof.

The methods for detecting polymorphisms can therefore involve
detecting an alteration in a nucleic acid. As used herein a "nucleic acid" is a
DNA or RNA molecule. A nucleic acid can be a segment of genomic DNA (e.g.,
an entire gene, an intron of a gene, an exon of a gene, a segment that includes
regulatory elements, a 5' non-coding segment, a 3' non-coding segment, or any
combination thereof). The nucleic acid can also be a cDNA (having exons but
not introns), an amplicon, an RNA, a primer, or a probe.

Probes and/or primers can be used that can hybridize to nucleic acid
segments flanking or including of any of SNPs, insertions, deletions,
polymorphic, or other variant segments of ADRB1, ADRB2, cytochrome P450
2D6 (CYP2D6), angiotensin converting enzyme (ACE), angiotensinogen,
angiotensin receptors, renin, Na⁺ channels (such as SCNNI A), adducin, sodium
(Na⁺) chloride (Cl⁻) co-transporters (such as SLC12A3), and/or WNK1 genes.
For example, probes and/or primers can be employed that hybridize to nucleic
acid segments flanking or including any of the following polymorphisms:
rs1801252 (ADRBl), rs1801253 (ADRBl), rs1042713 (ADRB2), rs1042714
(ADRB2), rs3892097 (CYP2D6), rs1799752 (ACE), rs699 (AGT), rs5186

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(AGT1R), rsl2750834 (renin), rs2228576 (SCNN1A), rs4961 (ADD1),
rs1529927 (SLC12A3), rs2107614 (WNK1), or rsl 159744 (WNK1). For
example, the probes and/or primers can separately hybridize to segments of any
of SEQ ID NO:2, 3, 6, 7, 10, 12, 14, 16, 19, 20, 22, 27, 30, 32, 33, 34, as well as
to the complementary sequences, amplicons, cDNA, cRNA, and genomic
sequences thereof. The probes and/or primers can hybridize to genomic,
complementary, amplicon, or cDNA sequences that flank up to 50 nucleotides of
any of SEQ ID NO:2, 3, 6, 7, 10, 12, 14, 16, 19, 20, 22, 27, 30, 33, or 34, on
either or both of the 5’ and 3’ sides of the polymorphism.

Methods and devices described herein can detect at least two of these
polymorphisms, or at least three of these polymorphisms, or at least four of
these polymorphisms, or at least five of these polymorphisms, or at least six
of these polymorphisms, or at least seven of these polymorphisms, or at least
eight of these polymorphisms, or at least nine of these polymorphisms, or at least
ten of these polymorphisms, or at least eleven of these polymorphisms, or at
least of twelve of these polymorphisms, or at least thirteen of these
polymorphisms, or at least fourteen of these polymorphisms, or at least fifteen of
these polymorphisms or all of these polymorphisms. In some embodiments, the
methods and devices described herein detect no other polymorphisms, although
such methods and devices can include steps and probes for detecting 1-4 control
target nucleic acids. For example, the methods, devices, and kits described
herein can detect and evaluate about sixteen polymorphisms.

The probes and primers can be of any convenient length selected by one
of skill in the art such as at least 12 nucleotides long, or at least 13 nucleotides
long, or at least 14 nucleotides long, or at least 15 nucleotides long, or at least 16
nucleotides long, or at least 17 nucleotides long, or at least 18 nucleotides long,
or at least 19 nucleotides long, or at least 20 nucleotides long. In some
embodiments, the probes and primers can be less than 150 nucleotides in length,
or less than 125 nucleotides in length, or less than 100 nucleotides in length, or
less than 75 nucleotides in length, or less than 65 nucleotides in length, or less
than 60 nucleotides in length, or less than 55 nucleotides in length, or less than
50 nucleotides in length, or less than 45 nucleotides in length, or less than 40
nucleotides in length.
To detect hybridization, it may be advantageous to employ probes, primers and other nucleic acids in combination with an appropriate detection means. Labels incorporated into primers, incorporated into the amplified product during amplification, or attached to probes that can hybridize to the target, or its amplified product, are useful in the identification of nucleic acid molecules. A number of different labels may be used for this purpose including, but not limited to, fluorophores, chromophores, radiolabels, enzymatic tags, antibodies, chemiluminescence, electroluminescence, and affinity labels. One of skill in the art will recognize that these and other labels can be used with success in this invention.

Examples of affinity labels include, but are not limited to the following: an antibody, an antibody fragment, a receptor protein, a hormone, biotin, dinitrophenyl (DNP), or any polypeptide/protein molecule that binds to an affinity label. Examples of enzyme tags include enzymes such as urease, alkaline phosphatase or peroxidase to mention a few. Colorimetric indicator substrates can be employed to provide a detection means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples. Examples of fluorophores include, but are not limited to, Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy2, Cy3, Cy5, 6-FAM, Fluorescein, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, ROX, TAMRA, TET, Tetramethylrhodamine, and Texas Red.

Means of detecting such labels are well known to those of skill in the art. For example, radiolabels may be detected using photographic film or scintillation counters. In other examples, fluorescent markers may be detected using a photodetector to detect emitted light. In still further examples, enzymatic labels are detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label or by use of spectrometer.

So called "direct labels" are detectable labels that are directly attached to or incorporated into a probe or primer, or to the target (sample) nucleic acid prior to hybridization to a probe that can, for example, be present on a plate,
chip, microtiter plate, or microarray. In contrast, so called "indirect labels" are joined to the hybrid duplex after hybridization. In some embodiments, the indirect label is attached to a binding moiety that has been attached to the target nucleic acid prior to the hybridization. Thus, for example, the target nucleic acid may be biotinylated before the hybridization. After hybridization, an avidin-conjugated fluorophore will bind the biotin-bearing hybrid duplexes providing a label that is easily detected. For a detailed review of methods of labeling nucleic acids and detecting labeled hybridized nucleic acids see, for example, Peter C. van der Vliet & Shiv Pillai, eds., Laboratory Techniques in Biochemistry and Molecular Biology (1993).

Probe arrays, assay plates, and gene chip technology provide a means of rapidly screening a large number of nucleic acid samples for their ability to hybridize to a variety of probes immobilized on a solid substrate that is part of the probe array, assay plate, gene chip or microarray. The technology capitalizes on the complementary binding properties of single stranded nucleic acid probe to screen nucleic acid samples by hybridization (Pease et al, Proc. Natl. Acad. Sci. U. S. A. 91: 5022-5026 (1994); U.S. Patent to Fodor et al. (1991)). Basically, a nucleic acid probe array or gene chip consists of a solid substrate with an attached array of single-stranded probe molecules. In some embodiments, the probes can fold back on (i.e., hybridize to) themselves to quench a signal from an attached label, but the probes unfold to hybridize to a target nucleic acid, whereupon the signal from the attached label becomes detectable. In other embodiments, the probe can be complementary to the segment of a target nucleic acid but the 3' end of the probe terminates one nucleotide short of a SNP in the target nucleic acid. The target nucleic acid can be longer than the probe. A signal can be detected upon primer extension of the probe, where the assay mixture contains just one type of labeled nucleotide that can base pair with the variant nucleotide of the SNP. After washing, the presence or absence of the SNP is detectable by incorporation or non-incorporation of the labeled SNP nucleotide into specific probes of the array or plate.

For screening, the chip, plate, or array is contacted with a nucleic acid sample (e.g., genomic DNA, cRNA, cDNA, or amplified copies thereof), which is allowed to hybridize under stringent conditions. The chip, plate, or array is then scanned to determine which targets have hybridized to which probes. The
probes are arrayed in known locations so a signal detected at a specific location indicates that its target has hybridized thereto.

Methods for directly synthesizing on or attaching nucleic acid probes to solid substrates are available in the art. See, e.g., U.S. Pat. Nos. 5,837,832 and 5,837,860, both of which are expressly incorporated by reference herein in their entireties. A variety of methods have been utilized to either permanently or removably attach the probes to the substrate. Exemplary methods include: the immobilization of biotinylated nucleic acid molecules to avidin/streptavidin coated supports (Holmstrom, (Anal. Biochem. 209: 278283 (1993)), the direct covalent attachment of short, 5'-phosphorylated primers to chemically modified polystyrene plates (Rasmussen et al, Anal. Biochem. 198: 138-142 (1991)), or the precoating of the polystyrene or glass solid phases with poly-L-Lys or poly L-Lys, Phe, followed by the covalent attachment of either amino- or sulfhydryl-modified oligonucleotides using bifunctional crosslinking reagents (Running et al, BioTechniques 8: 276 277 (1990); Newton, C. R. et al, Acids Res. 21: 1155-1162 (1993)). When immobilized onto a substrate, the probes are typically stabilized and therefore can be used repeatedly.

Hybridization can performed on an immobilized probe that is attached to a solid surface such as silicon, plastic, nitrocellulose, nylon or glass by addition of one or more target molecules. In some embodiments, the target nucleic acid can be attached to a solid surface and the probe can be added to the immobilized target nucleic acids. Numerous substrate and/or matrix materials can be used, including reinforced nitrocellulose membrane, activated quartz, activated glass, polyvinylidene difluoride (PVDF) membranes, polystyrene, polyacrylamide, poly(vinyl chloride), poly(methyl methacrylate), poly(dimethyl siloxane), photopolymers (which contain photoreactive species such as nitrenes, carbenes and ketyl radicals capable of forming covalent links with target molecules), and combinations thereof.

The term "hybridization" includes a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-
hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, primer extension, or the enzymatic cleavage of a polynucleotide by a ribozyme.

Hybridization reactions can be performed under conditions of different "stringency". The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another. Under low to medium stringent conditions, nucleic acid molecules at least 60%, 65%, 70%, 75% identical to each other remain hybridized to each other, whereas molecules with lower percent identity cannot remain hybridized. For detection of single base polymorphisms, higher stringency conditions can be used.

A preferred, non-limiting example of highly stringent hybridization conditions include hybridization in 6 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, more preferably at 60°C, and even more preferably at 65 °C.

When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary". A double-stranded polynucleotide can be "complementary" and/or "homologous" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second.

Complementarity" or "homology" (the degree that one polynucleotide is identical or complementary to another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to hydrogen bond with each other, according to generally accepted base-pairing rules.

**Detection / Identification of Genetic Variants in Expressed Polypeptides**

Genetic variants present in polypeptides such as ADRB1, ADRB2, cytochrome P450 2D6 (CYP2D6), angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptor, renin, Na+ channels (such as SCNN1A), adducin, sodium (Na+) chloride (Cl-) co-transporters (such as SLC12A3), and/or WNK1 can be detected by use of binding entities such as antibodies. Detection of specific differences in these polypeptides can be used to evaluate which blood pressure mediation is more effective.
Altered polypeptides can be detected in a selected fluid or tissue sample (e.g., cell scrapings, saliva, hair follicle, blood, skin tissue, or any convenient sample of a subject's nucleic acids). Any available methods for detecting polypeptides can be employed. Examples of such methods include immunoassay, Western blotting, enzyme-linked immunosorbant assays (ELISAs), radioimmunoassay, immunocytochemistry, immunohistochemistry, flow cytometry, immunoprecipitation, one- and two-dimensional electrophoresis, mass spectroscopy and/or detection of enzymatic activity.

Altered polypeptides can be detected by binding entities.

Antibodies and other binding entities can be used to detect genetic variants present in ADRBI, ADRB2, cytochrome P450 2D6 (CYP2D6), angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na\(^+\) channels (such as SCNN1 A), adducin, sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporters (such as SLC12A3), and/or WNK1 polypeptides. Such antibodies and binding entities can be prepared by available methods. For example, available amino acid sequences of non-variant and genetic variant ADRBI, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na\(^+\) channels (such as SCNN1 A), adducin, sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporters (such as SLC12A3), and/or WNK1, including those illustrated herein, can be used to make antibodies and binding entities. Suitable antibodies may include polyclonal, monoclonal, fragments (such as Fab fragments), single chain antibodies and other forms of specific binding molecules. Briefly, these polypeptide detection assays can include contacting a test sample with an antibody specific to the genetic variant site in the polypeptide, detecting the presence of a complex between the antibody and the polypeptide. In some embodiments, a signal from the polypeptide-antibody complex is detected.

Such antibody-based detection methods can any convenient immuno-detection method such as Western Blot, ELISA, radioimmunoassay, immunocytochemistry, immunohistochemistry, flow cytometry, and immunoprecipitation.

Antibodies can be used to detect or identify the presence of genetic variant forms of ADRBI, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na\(^+\) channels (such as
SCNN1A), adducin, sodium (Na') chloride (Cl') co-transporters (such as SLC12A3), and/or WNK1 polypeptides in a sample. The antibodies are specific for sites of genetic variations, and exhibit substantially no (or significantly less) binding to similar polypeptides that do not have the same genetic variation(s).

Generally speaking, such antibodies can be employed in any type of immunoassay, so long as the genetic variations in the polypeptides are reliably identified. This includes both the two-site sandwich assay and the single site immunoassay of the non-competitive type, as well as in traditional competitive binding assays.

For example, in a typical forward sandwich assay, unlabeled antibody is immobilized on a solid substrate, e.g., within microtiter plate wells, and the sample to be tested is brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex, a second antibody, labeled with a reporter molecule capable of emitting or inducing a detectable signal, is then added and incubation is continued allowing sufficient time for binding with the antigen at a different site and the formation of a ternary complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal, which may be quantified by comparison with a control sample containing known amounts of antigen.

Variations on the forward sandwich assay include the simultaneous assay, in which both sample and antibody are added simultaneously to the bound antibody, or a reverse sandwich assay in which the labeled antibody and sample to be tested are first combined, incubated and added to the unlabeled surface bound antibody. These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-site technique.

For the sandwich assays, the only limiting factor is that both antibodies have different binding specificities for the genetic variant polypeptide. Thus, a number of possible combinations are possible. For example, a primary antibody can bind specifically to the variant epitope of one of the variant polypeptides. A secondary antibody can bind to a different site on the genetic variant
polypeptide. As a more specific example, in a typical forward sandwich assay, a primary antibody is either covalently or passively bound to a solid support. The solid surface is usually glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinylchloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surfaces suitable for conducting an immunoassay.

Conventional antibody binding processes can be employed. Following binding, the solid phase-antibody complex is washed in preparation for the test sample. An aliquot of the test sample is then added to the solid phase complex and incubated at about 25° C for a period of time sufficient to allow binding of any genetic variant polypeptides present to the antibody. The primary antibody can bind specifically to the site of the genetic variant (e.g., the region of a variant amino acid and/or the structural changes associated therewith), but not to similar polypeptides that have no such genetic variant. After washing off unbound antibodies, the second antibody is then added to the solid phase complex and incubated at 25° C for an additional period of time sufficient to allow the second antibody to bind to the primary antibody-antigen solid phase complex (e.g., to a different site on the genetic variant polypeptide than is bound by the primary antibody). The second antibody may be linked to a reporter molecule, the visible signal of which is used to indicate the binding of the second antibody to any antigen in the sample.

As used herein, a "reporter molecule" or "label" is a molecule that provides an analytically detectable signal, allowing the detection of antigen-bound antibody. In some embodiments, detection is preferably at least relatively quantifiable, to allow determination of the amount of antigen in the sample, this may be calculated in absolute terms, or may be done in comparison with a standard (or series of standards) containing a known normal level of antigen. The term "label" is used interchangeably with "reporter molecule."

Many commonly used reporter molecules in this type of assay are either enzymes or fluorophores. In the case of an enzyme immunoassay an enzyme is conjugated to the second antibody, often by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are well known to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-
galactosidase and alkaline phosphatase, among others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitropheryl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labeled antibody is added to the first antibody-antigen complex and allowed to bind to the complex, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the tertiary complex of antibody-antigen-labeled antibody. The substrate reacts with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantified, usually spectrophotometrically, to give an evaluation of the amount of antigen that is present in the serum sample.

Additionally, fluorescent compounds, such as fluorescein or rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorophore-labeled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic longer wavelength. The emission appears as a characteristic color visually detectable with a light microscope. As in the enzyme immunoassay (EIA), the fluorescent-labeled antibody is allowed to bind to the first antibody-tagged polypeptide complex. After washing the unbound reagent, the remaining ternary complex is then exposed to light of the appropriate wavelength, and the fluorescence observed indicates the presence of the antigen.

Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent or bioluminescent molecules may also be employed. It will be readily apparent to the skilled artisan how to vary the procedure to suit the required use.

In another embodiment, the sample to be tested may be used in a single site immunoassay wherein it is adhered to a solid substrate either covalently or non-covalently. An unlabeled antibody is brought into contact with the sample bound on the solid substrate. After a suitable period of incubation, for a period of
time sufficient to allow formation of an antibody-antigen binary complex a
second antibody, labeled with a reporter molecule capable of inducing a
detectable signal, is then added and incubation is continued allowing sufficient
time for the formation of a ternary complex of antigen-antibody-labeled
antibody. For the single site immunoassay, the second antibody may be a general
antibody (i.e., xenogeneic antibody to immunoglobulin, particularly anti-(IgM
and IgG) linked to a reporter molecule) that is capable of binding an antibody
that is specific for the genetic variant polypeptide of interest.

Kits

Another aspect of the invention is one or more kits for evaluating blood
pressure from a test sample provided by, or obtained from, a subject.

The kits can include any reagents, components and instructions useful for
testing, assaying, detecting, identifying, and/or determining whether genetic
variations are present in ADRB1, ADRB2, CYP2D6, angiotensin converting
enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na⁺ channels
(such as SCNN1A), adducin, sodium (Na⁺) chloride (Cl⁻) co-transporters (such
as SLC12A3), and/or WNK1 polypeptides or nucleic acids that can be present in
the test samples.

The kits can include reagents, components and instructions for detecting,
identifying, and/or quantifying such polypeptides or nucleic acids. For example,
the kits may include primers, probes, labels, enzymes and/or other components
for detecting, and/or identifying genetic variations in such polypeptides or
nucleic acids.

In other embodiments, the kits may include one or more antibody
preparations that selectively bind to genetic variant ADRB1, ADRB2, CYP2D6,
angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors,
renin, Na⁺ channels (such as SCNN1A), adducin, sodium (Na⁺) chloride (Cl⁻)
co-transporters (such as SLC12A3), and/or WNK1 polypeptides, and a detection
means for detecting an antibody complex that can form (e.g., a label or reporter
molecule that is either bound to an antibody or is capable of binding to the
antibody).

One type of kit can include components for obtaining a sample from a
subject, and instructions for sample collection. For example, such a sample
collection kit can include one or more containers for sample collection such as
one or more vials, test tubes, or receptacles. The sample collection containers can include a solution for stabilizing samples placed in the containers. Such a stabilizing solution can include protease inhibitors, nuclease inhibitors, DNase inhibitors, RNase inhibitors, chelators, denaturants, salts, salts, and/or buffers.

The sample collection kit can also include components for sample collection such as swabs, droppers, syringes, needles, scalpels, and/or catheters. The instructions can include steps for sample collection, storage of the sample, and submission of the sample.

The kits can include one or more probes and/or primers each capable of specifically binding to a nucleic acid segment of at least 13, 14, 15, 16, 17, 18, 19, 20, or 25 nucleotides. In some embodiments, probes and/or primers are each capable of specifically binding to a nucleic acid segment of 15-30, 15-40, 15-50 nucleotides, or any number of nucleotides between 13-50 nucleotides, in a target DNA or RNA. The probes may be part of an array, microarray, microchip, assay plate, or nanochip. Alternatively, the probes or primers may be packaged separately and/or individually. In some embodiments, the probes or primers may be detectably labeled. For example, labels can be included on immobilized probes, where the label signals are quenched until hybridization occurs and then, upon hybridization, the label emits a detectable signal. Alternatively, one or more labels can be included in the kit that can bind to a hybridized complex between a probe and a target DNA or RNA.

Additional reagents can be included in the kits. For example, the kits may also contain reagents for detecting or identifying a genetic variant in an ADRB1, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na⁺ channels (such as SCNN1A), adducin, sodium (Na⁺) chloride (Cl⁻) co-transporters (such as SLC12A3), and/or WNK1 nucleic acid in a test sample. Such reagents can include reagents for isolating, storing and detecting nucleic acids. For example, the kits can include reagents and enzymes for nucleic acid amplification, primer extension, RNA reverse transcription, sequencing, restriction enzyme cleavage, and/or separation of nucleic acids. The kits may also include reagents such as solutions for stabilizing nucleic acids, solutions for purifying nucleic acids, nucleotide triphosphates, buffers, and/or other reagents that can be used in a test tissue sample.
Preservatives and/or antimicrobial agents can be included to stabilize reagents and prevent contamination, such as, for example, paraben, chlorobutanol, phenol sorbic acid, and the like.

It may also be desirable to include agents such as solvents for nucleic acids, reducing agents (e.g., beta-mercaptoethanol), stabilizing reagents (e.g., reagents for inhibiting nucleases, ribonucleases, disrupting tissues, precipitating nucleic acids, and the like).

In further embodiments, the kits can include a computer program product for use in conjunction with a computer system and the methods described herein. A computer program mechanism can be embedded in the computer program product. The computer program product can, for example, be a device with a computer program mechanism encoded thereon, where the computer program mechanism may be loaded into the memory of a computer and cause the computer to carry out at least one step of a method for assessing the malignant / benign status of a test thyroid tissue sample. For example, the device can be a computer readable storage medium, a flash memory, a compact disc (CD), a digital versatile disc, digital video disc, or an article of manufacture that tangibly includes one or more computer programs and memory storage. In some embodiments, the computer program product can be a computer readable storage medium. In such kits, the computer program mechanism can include instructions for determining, detecting, and/or identifying a genetic variant in an ADRB1, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na\(^+\) channel (such as SCNN1A), adducin, sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (such as SLC12A3), and/or WNK1 nucleic acid or polypeptide in a test sample.

In other embodiments, the kits can include a system, such as a computer, having a central processing unit and a memory coupled to the central processing unit. The memory may store instructions for determining, detecting, and/or identifying a genetic variant in an ADRB1, ADRB2, CYP2D6, angiotensin converting enzyme (ACE), angiotensinogen, angiotensin receptors, renin, Na\(^+\) channel (such as SCNN1A), adducin, sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (such as SLC12A3), and/or WNK1 nucleic acid or polypeptide in a test sample. The memory can also store therapeutic options for different genotyping results.
The kits can also include one or more therapeutic agents, for example, any blood pressure medications described herein.

**Definitions**

Some definitions are provided below; other definitions are provided in the other sections of the applications.

As used herein, "obtaining a test sample" involves removing a sample of tissue from a patient, receiving a sample of tissue from a patient, receiving a patient's tissue sample from a physician, receiving a patient's tissue sample via mail delivery and/or removing a patient's tissue sample from a storage apparatus (e.g., a refrigerator or freezer) or a facility. Thus, obtaining a test sample can involve removal or receipt of the test sample directly from the patient, but obtaining a test sample can also include receipt of a test sample indirectly from a medical worker, from a storage apparatus/facility, from a mail delivery service after transportation from a medical facility, and any combination thereof. The test sample can therefore originate in one location, and be transported to another location where it is received and tested. Any of these activities or combinations of activities involves "obtaining a test sample."

As used herein a probe refers to a single DNA or RNA molecule (a nucleic acid oligomer) or a collection of nucleic acid molecules (nucleic acid oligomers) where the DNA molecules have at least one segment with a sequence that is complementary to a region of a target nucleic acid. The probe can hybridize with the target nucleic acid under stringent conditions. In some cases, the probe can hybridize with the target nucleic acid under highly stringent conditions. The probe is not identical to naturally available nucleic acids because it has additional components such as one or more labels, one or more (engineered) restriction sites, one or more molecular barcodes, one or more tags for identification or retrieval of the probe (e.g., with or without the target hybridized thereto). In some instances the probe is attached to a solid surface such as a chip, an array, a bead, or other surface.

As used herein a primer contains a region that is designed to hybridize to a targeted locus (e.g., a targeted polymorphic locus or a nonpolymorphic locus). The primer and may contain a priming sequence designed to allow PCR amplification. The primer can have at least one segment with a sequence that is complementary to a region of a target nucleic acid. The primer can hybridize
with the target nucleic acid under stringent conditions. In some cases, the primer can hybridize with the target nucleic acid under highly stringent conditions. The primer is not identical to naturally available nucleic acids because has additional components such as a molecular barcode, a tag, an engineered restriction site, or a combination thereof. A primer may contain a random region that differs for each individual molecule. The terms "test primer" and "candidate primer" are not meant to be limiting and may refer to any of the primers disclosed herein.

As used herein a "binding entity" is a molecule or molecular complex that can recognize and bind to selected target molecules. Such binding entities can be antibodies or any molecule that has a binding domain for a target molecule.

A number of proteins can serve as protein scaffolds to which binding domains for targets can be attached and thereby form a suitable binding entity. The binding domains bind or interact with the targets of the invention while the protein scaffold merely holds and stabilizes the binding domains so that they can bind. A number of protein scaffolds can be used. For example, phage capsid proteins can be used. See Review in Clackson & Wells, Trends Biotechnol. 12:173-184 (1994). Phage capsid proteins have been used as scaffolds for displaying random peptide sequences, including bovine pancreatic trypsin inhibitor (Roberts et al, PNAS 89:2429-2433 (1992)), human growth hormone (Lowman et al, Biochemistry 30:10832-10838 (1991)), Venturini et al, Protein Peptide Letters 1:70-75 (1994)), and the IgG binding domain of Streptococcus (OTSTeil et al, Techniques in Protein Chemistry V (Crabb, L., ed.) pp. 517-524, Academic Press, San Diego (1994)). These scaffolds have displayed a single randomized loop or region that can be modified to include binding domains for selected targets.

Researchers have also used the small 74 amino acid a-amylase inhibitor Tendamistat as a presentation scaffold on the filamentous phage M13. McConnell, S.J & Hoess, R.H., J. Mol. Biol. 250:460-470 (1995). Tendamistat is a β-sheet protein from *Streptomyces tendae*. It has a number of features that make it an attractive scaffold for binding peptides, including its small size, stability, and the availability of high resolution NMR and X-ray structural data. The overall topology of Tendamistat is similar to that of an immunoglobulin domain, with two β-sheets connected by a series of loops. In contrast to
immunoglobulin domains, the β-sheets of Tendamistat are held together with two rather than one disulfide bond, accounting for the considerable stability of the protein. The loops of Tendamistat can serve a similar function to the CDR loops found in immunoglobulins and can be easily randomized by in vitro mutagenesis. Tendamistat is derived from Streptomyces tendae and may be antigenic in humans. Hence, binding entities that employ Tendamistat are preferably employed in vitro.


The following non-limiting examples further illustrate aspects of the invention.

**Example 1: Sample Processing**

Each patient is given a collection kit consisting of two buccal swabs and two uniquely barcoded tubes (termed A and B swabs) containing a proprietary lysis buffer consisting of 50mM Tris pH 8.0, 50mM EDTA, 25mM Sucrose, 100mM NaCl, and 1 % SDS. The patient will use the swab to collect buccal cells by scraping the inside of their cheek and place the swab in the provided barcoded tube, one swab for each cheek. Once the swab has been placed into the lysis buffer the cells are no longer viable and therefore samples are now considered to be nucleic acids and safe to be shipped via standard mail. All samples are checked-in. The barcodes of the samples are scanned and their arrival in the laboratory is confirmed.
FIGs. 3A-3B show schematic diagrams illustrating slight variations in sample processing. In general, two samples (Swab A and Swab B) are taken. The Swab A sample is subjected to the process (DNA Extraction through Assays, Genomic Analysis, or the PCR QA Assay. If such failure occurs, then the other sample (Swab B) is subjected to the process, as illustrated in FIGs. 3A and/or 3B.

The samples are grouped into sets of 91 and assigned positions in 96 sample grids (12 x 8 grid layout) for DNA extraction. The remaining five positions in each grid can be extraction controls (four negative controls [H₂O] and one non-human positive). The five controls can be assigned random positions in each grid, giving each grid/plate a unique "plate fingerprint." The randomly assigned controls prevent possible plate swaps or 180° rotations as every plate is now identifiable simply by control positions. All samples are then normalized to a volume of 650ul by addition of the above mentioned lysis buffer. Additionally, 25ul of proteinase K (ProK) is added and each sample is incubated in a 55 °C oven for a minimum of 4 hours.

Following such incubation, the samples are extracted using a BioSprint96 (KingFisher96) Robotic workstation with magnetic-particle DNA purification chemistry to isolate genomic DNA (GenomicDNA) from tissue samples. This protocol utilizes the chemistry from the eVoMagDNA Extraction KF96 Kit (Verde Labs, Marietta, GA) and is run to specifications provided by the manufacturer.

Following DNA extraction and subsequent desiccation, the DNA is resuspended in HPLC water. Five microliters of each sample is then aliquoted to assay plates for the first pair of QA assays, both a PicoGreen fluorometric quantification and a spectrophotometric purity estimation. The fluorescence and absorbance data is analyzed for all samples in the 96 well plate, including the five controls. The positions of the negative controls is confirmed and accessed for possible plate contamination. The results for the positive control as well as the samples on the plate are analyzed for quality metrics using a systems analysis approach. The outliers are statistically assessed. After the quantification and purity evaluations, QA assay robotic systems are used to transfer the samples into racks of 96 sample septa sealed plates (to ensure there is no
evaporative loss) and a fractional volume of each sample is used to create a
daughter plate of the samples at a normalized concentration of 5 ng/μl for the
PCR QA assays and subsequent genotyping. The creation of the normalized
daughter plate serves two purposes. First, it allows the immediate storage of the
primary stock of each sample at -80 °C avoiding the need for unnecessary freeze-
thaw of samples and the potential contamination risks associated with repeated
accessing of the stock. Second, it avoids unnecessary waste of the DNA
associated with the use of full concentration stock for the PCR applications (this
-80 °C stock DNA can be used at any time or saved for future testing).

5

Any samples that fail any of the QA assays can re-enter the pipeline and
be sorted and re-processed from the B-swab, which is the second tube/swab in
the kit sent to the customer mentioned above. By always having a backup sample
it is not necessary to go back to the customer to ask for a re-swab. If the
quantity and purity are still insufficient then whole genome amplification and/or
organic re-extraction can be employed.

10 Following the passage of the QA thresholds normalized fractions of the
samples are transferred to PCR plates for genotyping. Each sample is analyzed
using three different methodologies, the Sequenom MassArray genotyping
platform, Sanger sequencing using the ABI 3730x1 genomic analyzer from
Applied Biosystems, and classical PCR and gel sizing to determine
insertion/deletion status. The Sequenom MassArray genotyping platform is used
to analyze the following SNP sites: rs1042713, rs1042714, rs1 59744,
rs12750834, rs1801252, rs1801253, rs2107614, rs227869, rs4244285, rs4961,
and rs699. Sanger sequencing is used to analyze the following SNPs:

15 rs3892097, rs3758581, rs2228586, and rs5186. Finally classical gel sizing is
used to determine the insertion/deletion status of the rs1799752 SNP.

Example 2: Sequenom MassArray Assay design and Processing

The Sequenom platform is able to perform genotyping as a twelve-plex
assay (testing 12 variable sites in one reaction) in a 96 well format using one
aliquot of DNA. The AssayDesign software from Sequenom is used to generate
both PCR and single base extension primers using the individual rs number of
each variable site to create the final assay design. Table 1 shows examples of
primers that can be used to detect various single nucleotide polymorphisms.
Table 1: Primers for Amplification of Nucleic Acid Variant Segments

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>2nd PCRP</th>
<th>SEQ ID</th>
<th>1st PCRP</th>
<th>SEQ ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1042714</td>
<td>ACGTTGGATGAGCACATGACGATGCCATGC</td>
<td>NO: 36</td>
<td>ACGTTGGATGAGCACCTTCTGCTGGAC</td>
<td>NO: 37</td>
</tr>
<tr>
<td>rs699</td>
<td>ACGTTGGATGCTGAGACGATGCCATGC</td>
<td>NO: 38</td>
<td>ACGTTGGATGTTGACGATGATGCC</td>
<td>NO: 39</td>
</tr>
<tr>
<td>rs4961</td>
<td>ACGTTGGATGCTGAGACGATGCCATGC</td>
<td>NO: 40</td>
<td>ACGTTGGATGAGCATAGGCTGAACGCTG</td>
<td>NO: 41</td>
</tr>
<tr>
<td>rs12750834</td>
<td>ACGTTGGATGAGACGATGCCATGC</td>
<td>NO: 42</td>
<td>ACGTTGGATGACAGCTTCGCTTACCTG</td>
<td>NO: 43</td>
</tr>
<tr>
<td>rs1801252</td>
<td>ACGTTGGATGAGACGATGCCATGC</td>
<td>NO: 44</td>
<td>ACGTTGGATGACGATGCCACATGCGCC</td>
<td>NO: 45</td>
</tr>
<tr>
<td>rs1159714</td>
<td>ACGTTGGATGAGACGATGCCATGC</td>
<td>NO: 56</td>
<td>ACGTTGGATGACGATGCCACATGCGCC</td>
<td>NO: 57</td>
</tr>
</tbody>
</table>

rsll59744  | ACGTTGGATGAGACGATGCCATGC      | NO: 58 | ACGTTGGATGAGACGATGCCACATGCGCC | NO: 59 |
DNA samples at a concentration of 5ng/ul undergo a PCR using the above designed PCR primers and the Sequenom iPLEX Gold Reagent kit under the conditions described in Table 2.

**Table 2: PCR Reaction Mixture**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Final Concentration</th>
<th>Vol/rxn (uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, HPLC</td>
<td>N/A</td>
<td>1.8</td>
</tr>
<tr>
<td>10x PCR Buffer with 20mM MgCl₂</td>
<td>2mM MgCl₂</td>
<td>0.5</td>
</tr>
<tr>
<td>25mM MgCl₂</td>
<td>2mM</td>
<td>0.4</td>
</tr>
<tr>
<td>25mM dNTP Mix</td>
<td>500uM</td>
<td>0.1</td>
</tr>
<tr>
<td>0.5mM Primer Mix</td>
<td>0.1uM</td>
<td>1</td>
</tr>
<tr>
<td>5U/uL PCR Enzyme</td>
<td>1 unit</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Volume: 4

10ng/uL DNA: 10ng/rxn

Total Volume: 5

The PCR reaction cycling conditions can be as illustrated in Table 3.

**Table 3: PCR Reaction Cycling**

<table>
<thead>
<tr>
<th>Cycler Program iPLEX- PCR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (°C)</td>
<td>Time (min)</td>
</tr>
<tr>
<td>95</td>
<td>2:00</td>
</tr>
<tr>
<td>95</td>
<td>0:30</td>
</tr>
<tr>
<td>56</td>
<td>0:30</td>
</tr>
<tr>
<td>72</td>
<td>1:00</td>
</tr>
<tr>
<td>72</td>
<td>5:00</td>
</tr>
<tr>
<td>4</td>
<td>∞</td>
</tr>
</tbody>
</table>

Directly following PCR amplification, excess primers and deoxynucleotide triphosphates are removed via a SAP (shrimp alkaline phosphatase) reaction under the conditions described in Table 4.
The Shrimp Alkaline Phosphatase reaction is incubated at 37 °C for 40 min, followed by incubation at 85 °C for 5 min. The samples can be stored at 4 °C indefinitely.

After the SAP reaction is completed the samples can be subjected to single base extension reactions using the primers described in Table 5, and the conditions described in Table 6 and 7.

### Table 4: PCR Clean-Up

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Final Concentration</th>
<th>Vol/rxn (uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, HPLC</td>
<td>N/A</td>
<td>1.53</td>
</tr>
<tr>
<td>SAP Buffer (10x)</td>
<td>0.24x</td>
<td>0.17</td>
</tr>
<tr>
<td>5U/uL PCR Enzyme</td>
<td>1 unit</td>
<td>0.2</td>
</tr>
<tr>
<td>Volume</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>PCR product</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Single Base Extension Primers

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sequence</th>
<th>SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1042714</td>
<td>ACACCTCGTCCCTTT</td>
<td>60</td>
</tr>
<tr>
<td>rs699</td>
<td>CTGGCTGCTCCCTGA</td>
<td>61</td>
</tr>
<tr>
<td>rs4961</td>
<td>ACTGCTTCCATCTCAGG</td>
<td>62</td>
</tr>
<tr>
<td>rs12750834</td>
<td>AGTCTCTGTAAATGCCG</td>
<td>63</td>
</tr>
<tr>
<td>rs1801252</td>
<td>GTGCCCTCCCAGGCACCGA</td>
<td>64</td>
</tr>
<tr>
<td>rs1801253</td>
<td>CGCGCGAGGAGAGCAGT</td>
<td>65</td>
</tr>
<tr>
<td>rs227869</td>
<td>AGCTTGATCTGCCTCATTCA</td>
<td>66</td>
</tr>
<tr>
<td>rs2107614</td>
<td>TCCTCCAAAAAAAGAAAAC</td>
<td>67</td>
</tr>
<tr>
<td>rs1529927</td>
<td>GTTACCGACATCCCGCATATTG</td>
<td>68</td>
</tr>
<tr>
<td>rs4244285</td>
<td>TAAAGTAGAGTGTTATGGGTTC</td>
<td>69</td>
</tr>
<tr>
<td>rs1042713</td>
<td>GGAGGGGTTCCGGCGCATGGCTTC</td>
<td>70</td>
</tr>
<tr>
<td>rs1159744</td>
<td>CAAATGTTAAGTGATAGAAAATTTTA</td>
<td>71</td>
</tr>
</tbody>
</table>

### Table 6: Single Base Extension Reaction Conditions

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Final Concentration</th>
<th>Vol/rxn(uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, HPLC</td>
<td>N/A</td>
<td>0.619</td>
</tr>
<tr>
<td>iPlex Gold Buffer</td>
<td>0.222x</td>
<td>0.200</td>
</tr>
<tr>
<td>iPlex Termination Mix</td>
<td>1x</td>
<td>0.200</td>
</tr>
<tr>
<td>iPlex Extend Primer Mix</td>
<td>varies</td>
<td>0.940</td>
</tr>
<tr>
<td>iPlex Enzyme</td>
<td>1x</td>
<td>0.041</td>
</tr>
<tr>
<td>Volume</td>
<td></td>
<td>2.000</td>
</tr>
</tbody>
</table>
Reagents | Final Concentration | Vol/rxn (uL)
---|---|---
PCR product | | 7
Total Volume | | 9

Table 7: Single Base Extension Reaction Cycling conditions

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>0:30</td>
</tr>
</tbody>
</table>
| 94 | 0:05 | 40 cycles
| 52 | 0:05 | 5 cycles ↓
| 80 | 0:05 | ↓
| 72 | 3:00 | 
| 4 | forever | 

After completion of all of the above reactions, the samples are run through resin based clean-up to remove excess salts according to standard Sequenom protocols. The samples are then spotted onto the Sequenom provided SpectroChip using the Sequenom Nanodispenser according to manufacturer protocols and subsequently processed on the Sequenom MALDI-TOF platform.

A sample results report is provided in Table 7. The two letters for each polymorphism type are for the two alleles present in the subjects, illustrating that the subjects are homozygous for some polymorphisms (e.g., subject GCE0104 is homozygous (G/G) for the variable site in the rs042713 polymorphism, but subject GCE0120 is heterozygous (GA) for that site).

Table 8: Results

<table>
<thead>
<tr>
<th>SNP</th>
<th>GCE0120</th>
<th>GCE0104</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1042713</td>
<td>GA</td>
<td>GG</td>
</tr>
<tr>
<td>rs1042714</td>
<td>GC</td>
<td>GG</td>
</tr>
<tr>
<td>rs1159744</td>
<td>AA</td>
<td>AT</td>
</tr>
<tr>
<td>rs12750834</td>
<td>GA</td>
<td>GG</td>
</tr>
<tr>
<td>rs1529927</td>
<td>GG</td>
<td>GG</td>
</tr>
<tr>
<td>rs1801252</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>rs1801253</td>
<td>GG</td>
<td>GG</td>
</tr>
<tr>
<td>rs2107614</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>rs227869</td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>rs4244285</td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>rs4961</td>
<td>GG</td>
<td>GG</td>
</tr>
<tr>
<td>rs699</td>
<td>CG</td>
<td>GG</td>
</tr>
</tbody>
</table>
Example 3: Sanger Sequencing primer design and workflow

All primers for Sanger sequencing were designed using the free, web-based primer design tool Primer3. 500 base pairs of flanking sequence for each SNP (single nucleotide polymorphism) were entered into Primer3. The top primer candidate for each site was chosen and optimized using a buffer panel with varying MgCl\textsubscript{2} concentrations and a temperature gradient to determine the optimal cycling conditions for each primer pair.

Table 9: Primers for Sequencing of SNPs

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
<th>SEQ ID</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3892097 F</td>
<td>TTCAGTCCCTCCTGAGCTA</td>
<td>NO:72</td>
<td>SNP</td>
</tr>
<tr>
<td>rs3892097 R</td>
<td>AAGGTGGATGCACAAAGAG</td>
<td>NO:73</td>
<td>SNP</td>
</tr>
<tr>
<td>rs3758581 F</td>
<td>GTGCATCTGAGCAGTCCTC</td>
<td>NO:74</td>
<td>SNP</td>
</tr>
<tr>
<td>rs3758581 R</td>
<td>CCAACTGGAATCAACAGAA</td>
<td>NO:75</td>
<td>SNP</td>
</tr>
<tr>
<td>rs2228586 F</td>
<td>GAAGTGTTCTCGTCTAGCAA</td>
<td>NO:76</td>
<td>SNP</td>
</tr>
<tr>
<td>rs2228586 R</td>
<td>CAGAGAGAGAGTCCCATTT</td>
<td>NO:77</td>
<td>SNP</td>
</tr>
<tr>
<td>rs5186 F</td>
<td>CCACCTCAACCTTTCAACAA</td>
<td>NO:78</td>
<td>SNP</td>
</tr>
<tr>
<td>rs5186 R</td>
<td>TGGACAGAACAATCTGGAAAC</td>
<td>NO:79</td>
<td>SNP</td>
</tr>
</tbody>
</table>

The region encompassing the SNP was amplified from sample nucleic acids by PCR using optimized individual cycling conditions for each SNP site. Directly after PCR amplification each sample is cleaned up using a size exclusion micro-filtration plate from Millipore and entered into the Sanger sequencing reaction. Each sample is sequenced in both the forward (3') and reverse (5') direction giving double conformation of the allelic state. These forward and reverse sequences from each patient are then aligned to the human reference sequence using the CLC DNA workbench program creating an alignment file from which the allele call is made and added to the final SNP call report.

FIG. 6 illustrates the results from one such alignment.

Example 4: Gel Sizing primer design and workflow

To accurately call the insertion/deletion status for site rsL799752, PCR amplification of sample nucleic acids is performed followed by gel electrophoresis. The PCR primers for this site were also designed and optimized using Primer3 and the above mentioned buffer and temperature gradient. The following primer sequences and PCR conditions were ultimately chosen:
Table 10: Primer Sequences for PCR of rs799752 Insertion / Deletion

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
<th>SEQ ID</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799752_F-2</td>
<td>CCCATTTCTCTAGACCTGCT</td>
<td>NO:80</td>
<td>INDEL</td>
</tr>
<tr>
<td>rs1799752_R-2</td>
<td>GGGATGGTGTCGCCTACATA</td>
<td>NO:81</td>
<td>INDEL</td>
</tr>
</tbody>
</table>

Following PCR amplification, each sample is loaded into its own well of a 2% agarose gel and run at 150 mV for approximately 45 min and stained in a bath of GelRed for 2 hours prior to imaging with UV light. The resulting image is used to score the presence or absence of a 288bp ALU visually by examining the gel for either the higher molecular weight band (indicating the presence of the 288bp ALU), the lower molecular weight band (indicating the absence of the 288bp ALU) or both (indicating a heterozygous state. A sample image of the gel is provided in FIG. 7.

Example 5: Genotyping Reports

Once all tests are performed a report is generated containing all results for each tested patient. One example of a report for subjects GCE0120 and GCE0104, is shown below. The two letters for each polymorphism type are for the two alleles present in the subjects, illustrating that the subjects are homozygous whereas other subjects are heterozygous for the variable site of each polymorphism.

Table 11: Results from Analysis of Polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism type</th>
<th>GCE0120</th>
<th>GCE0104</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sequenom Results</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1042713</td>
<td>GA</td>
<td>GG</td>
</tr>
<tr>
<td>rs1042714</td>
<td>GC</td>
<td>GG</td>
</tr>
<tr>
<td>rs1159744</td>
<td>CG</td>
<td>CG</td>
</tr>
<tr>
<td>rs12750834</td>
<td>GA</td>
<td>GG</td>
</tr>
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<tr>
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<tr>
<td>rs699</td>
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</table>
Polymorphism type | GCE0120 | GCE0104
--- | --- | ---
Sanger Sequencing Results
rs3892097 | CC | CC
rs3758581 | GG | GG
rs3758580 | CC | CT
rs2228586 | TT | TT
rs5186 | AC | AA

Gel Results
rs1799752 | +/+ | +/-

Example 6: Clinical Study Protocol

Clinical Protocol Summary

| Study Title: | Assessment of the Relationship between Genes that Encode Proteins Important in Blood Pressure Regulation and Blood Pressure Therapy in Patients with Hypertension. |
| Study Device: | The Geneticure Pharmacogenetic Testing Kit. The kit contains two buccal swabs with two buffer solution vials to stabilize DNA. These buccal swabs are used for DNA collection which is then extracted for analysis of genes important in high blood pressure. |
| Target Indication for Use: | The Geneticure Pharmacogenetic Testing Kit is a pharmacogenomic treatment decision support product that tests for clinically important genetic variants affecting a patient’s response to antihypertensive medications. |
| Study Design: | This is a post-hoc association study of patients who have been diagnosed with high blood pressure and have been stable on medication treatment for at least 6 months. |
| Study Population: | To be enrolled in this study, subjects must meet ALL of the inclusion criteria and NONE of the exclusion criteria: |
| Inclusion Criteria | 1. Subject is able and willing to provide informed consent
2. Subject is ≥ 30 and ≤ 70 years of age
3. Subject with diagnosis of Hypertension for a minimum of 1 year
4. Subject has been on the same class/classes of blood pressure medication for a minimum of 6 months. |
Note: A change in dosage, frequency, or specific medication is acceptable as long as there have been no changes to the class/classes of medications prescribed.

5. Subject with a Body Mass Index (BMI) \( \geq 19 \) and \( \leq 35 \)

6. Subject is currently prescribed and taking one of the following classes of medications alone or in combination with each other or a Ca+ channel blocker.
   - Diuretics
   - ACE Inhibitors
   - Angiotensin Receptor Blocker (ARB)
   - Beta-blockers

Exclusion Criteria

1. Subject has clinically significant kidney disease as determined by the investigator.
2. Subject has clinically significant cardiac disease as determined by the investigator.
3. Subject has clinically significant vascular disease as determined by the investigator.
4. Subject has a diagnosis of secondary hypertension or is experiencing a complication of pregnancy.
5. Subject is currently prescribed and taking any additional class of medication(s) for high blood pressure not included in the list above, with the exception of a Ca+ channel blocker.
6. Subject has Systolic BP \( > 190 \) or Diastolic BP \( > 120 \) documented within the six months prior to visit.
7. Subject has a regular alcohol intake of greater than 2.1 units per week in the past 6 months
8. Subject has smoked greater than two packs of cigarettes (total) or equivalent nicotine intake in the past 6 months.
9. Subject has an anticipated survival less than 12 months.
10. Any other reason that the subject is inappropriate for study enrollment in the opinion of the Investigator.

| Primary Study Objective: | To assess the relationship between the drug therapy class/combination of therapy classes that resulted in the best blood pressure control for a patient vs. what the Geneticure high blood pressure panel would have predicted. |
Secondary Study
Objectives:
- To assess the clinical time to achieve optimal blood pressure treatment.
- To assess the number of office visits required to achieve optimal blood pressure treatment.

1 Introduction

Hypertension (high blood pressure) is one of the most important preventable contributors to disease and death in the United States and represents the most common condition seen in the primary care setting (The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. Arch Intern Med. 157(21):2413-2446 (1997); Chobanian et al. JAMA 289(19):2560-2572 (2003)). According to the American Heart Association, approximately 78 million adults (1 in 3) living in the United States have hypertension with more than 5 million new diagnoses made each year (American Heart Association. Heart and Stroke Statistics - 2004 Update. Paper presented at: Dallas: American Heart Association (2004); Roger et al. Circulation. 125(1):e2-e220 (2012)). Of these individuals, 82% are aware they have it, 75% are currently being treated for it, but only 52% have their blood pressure under control. Thus, about 48% of individuals with hypertension do not have adequate blood pressure control. Hypertension is known to lead to myocardial infarction (heart attack), stroke, renal failure, and death if not detected early and treated appropriately. In fact, in 2009, high blood pressure was listed as a primary or contributing cause of death in ~350,000 of the ~2.4 million U.S. deaths (14% of all deaths). From 1999-2009 the number of deaths attributable to hypertension increased by 44%.

Refractory (or resistant) hypertension is defined as blood pressure that remains above clinical guideline goals in spite of concurrent use of three antihypertensive agents of different classes (Akpunonu et al, Disease-a-month : DM. Oct 1996;42(10):609-722). Critically, refractory hypertension is noted in approximately 25-30% of all individuals being treated for hypertension. Refractory hypertension is a common clinical problem which contributes to the high levels of morbidity and mortality. The inability to gain control of blood pressure in these individuals may be related to the pharmacogenetics of the individual coupled with the specific classes of drugs and/or combination of
classes chosen for that individual (Calhoun et al. Circulation 117(25):e5 10-526 (2008); Johnson & Turner, Curr Opin Mol Ther 7(3):218-225 (2005)). In 2009, the direct and indirect economic burden on the United States health care system associated with hypertension was estimated at $51 billion. With the advent of improved diagnostic techniques, increased rates of health care utilization and screening, and the increasing age of the population, a continual upward trend in this expenditure is expected.

Globally, nearly 1 billion individuals have been diagnosed with hypertension with an estimate of an additional 400 million living with undiagnosed hypertension. Hypertension is the leading cause of premature death and the leading cause of cardiovascular disease worldwide. Similar to the continued upward trend in prevalence as seen in the United States, it is estimated that in 2025 approximately 1.56 billion adults will be living with hypertension. Because nearly two-thirds of the people living with hypertension worldwide reside in developing countries, providing optimal treatment at the lowest cost is critically important.

Unfortunately, despite a significant impulse in the medical community to move towards an "individualized medicine" approach to patient centered treatment, the current clinical treatment strategy is based on a set algorithm which does not take into account individual patient differences. Rather, physicians are guided to choose a drug (one out of many options) in a given class of drugs and use that specific drug as a "first line therapy" (typically initiating with the diuretic class) and titrate that specific drug of choice to therapeutic dosage regardless of efficacy (Chobanian et al. JAMA 289(19):2560-2572 (2003)). It is only after a prolonged course of treatment with that specific class of drug that clinical efficacy is determined (typically three months). At this stage, if clinical guideline goals for blood pressure have not been met, it is often recommended that the patient remain on the "first line therapy" whilst an additional drug from a different class of drugs (typically an Angiotensin converting enzyme inhibitor (ACE inhibitor) or Angiotensin II receptor blocker (ARB)) is added to the pharmacologic regimen. Again, this drug is titrated to recommended therapeutic dosage and another prolonged course of treatment is initiated before clinical efficacy is determined (an additional three months - six months since initiation of treatment). If at this point, clinical guideline goals for
blood pressure have not been met, a third drug from a third class of drugs (typically a beta-blocker) is added and the process is repeated (another three months - nine months from initiation of treatment). Further, if clinical guideline goals have continued to be elusive, the diagnosis of refractory hypertension is added and the process is reinitiated with a different combination of drugs, different classes of drugs, different drug options within a given class of drugs, different dosages, or all of the above. Thus, from the time of initial diagnosis and the start of treatment to the point in which blood pressure is adequately controlled may take anywhere from three months to well over one year. This trial-and-error standard of care is clearly not optimal.

The blood pressure panel created by Geneticure has been created to comprehensively assess fourteen common genetic variants in the cardiac, vascular, and renal systems that can improve therapeutic guidance for the clinician based on known functional alterations of the protein through these genetic changes, as well as demonstrated effects of certain drug classes on these various genotypes. Based on this information, a clinician can guide therapy with knowledge specific to their patient, rather than "trial-and-error" based on population data and using drugs with least side effects initially.

1.1 Investigational Device: Geneticure Pharmacogenetic Testing Kit

The Geneticure pharmacogenetic testing kit contains two buccal swabs with two buffer solution vials to stabilize DNA. These buccal swabs are used for DNA collection which is then extracted for analysis of genes important in high blood pressure.

The Geneticure Pharmacogenetic Testing Kit is a pharmacogenomic treatment decision support product that tests for clinically important genetic variants affecting a patient’s response to antihypertensive medications.

1.2 Genetic Analysis

Each sample can be analyzed for fourteen common genetic variants using 3 different methodologies, the Sequenom MassArray genotyping platform, Sanger sequencing using the ABI 3730x1 genomic analyzer from Applied
Biosystems, and classical PCR and gel sizing to determine insertion/deletion status (see, FIGs. 3A-3B).

2 Methodology

2.1 Study Design and Protocol Overview

This is a post-hoc association study of patients who have been diagnosed with high blood pressure and have been stable on medication treatment for at least 6 months. The purpose of this study is to evaluate the relationship between optimal medication therapy (or the therapy that has resulted in the most stable blood pressure for that particular patient) and the predicted optimal medication therapy based on a patient's genetic profile.

Chart reviews for the patient's history of antihypertensive therapy can be coupled with buccal swabs and blood pressure readings collected from eligible patients who have provided informed consent. The swab can be analyzed for fourteen genetic variants that are associated with antihypertensive therapy response (efficacy, side-effects).

2.2 Study Objective

To assess the relationship between the drug therapy class/combination of therapy classes that resulted in the best blood pressure control for a patient vs. what the Geneticure high blood pressure panel would have predicted.

2.3 Secondary Objectives

The secondary objectives are as follows:

• To assess the clinical time to achieve optimal blood pressure treatment.
• To assess the number of office visits required to achieve optimal blood pressure treatment.

3 Investigational Study Center

This study will be conducted at up to 5 study centers within the United States that have adequate resources for trial responsibilities.

4 Study Population

To be enrolled in this study, subjects must meet ALL of the inclusion criteria and NONE of the exclusion criteria:
4.1 Inclusion Criteria

1. Subject is able and willing to provide informed consent
2. Subject is ≥ 30 and ≤ 70 years of age
3. Subject with diagnosis of Hypertension for a minimum of 1 year
4. Subject has been on the same class/classes of blood pressure medication for a minimum of 6 months. Note: A change in dosage, frequency, or specific medication is acceptable as long as there have been no changes to the class/classes of medications prescribed.
5. Subject with a Body Mass Index (BMI) ≥ 19 and ≤ 35
6. Subject is currently prescribed and taking one of the following classes of medications alone or in combination with each other or a Ca+ channel blocker.
   - Diuretics
   - ACE Inhibitors
   - Angiotensin Receptor Blocker (ARB)
   - Beta-blockers

4.2 Exclusion Criteria

1. Subject has clinically significant kidney disease as determined by the investigator.
2. Subject has clinically significant cardiac disease as determined by the investigator.
3. Subject has clinically significant vascular disease as determined by the investigator.
4. Subject has a diagnosis of secondary hypertension or is experiencing a complication of pregnancy.
5. Subject is currently prescribed and taking any additional class of medication(s) for high blood pressure not included in the list above, with the exception of a Ca+ channel blocker.
6. Subject has Systolic BP > 190 or Diastolic BP > 120 documented within the six months prior to visit.
7. Subject has a regular alcohol intake of greater than 21 units per week in the past 6 months
8. Subject has smoked greater than two packs of cigarettes (total) or equivalent nicotine intake in the past 6 months.
9. Subject has an anticipated survival less than 12 months.
10. Any other reason that the subject is inappropriate for study enrollment in the opinion of the Investigator.
5 **Informed Consent**

The investigator will prepare an informed consent form in accordance with this study protocol and all regulatory requirements (21 CFR Part 50) using the template informed consent form provided by the sponsor. The informed consent form must be submitted to the IRB and a copy of the final IRB-approved consent form must be submitted to the Study Management Center prior to the start of the study at that investigational site.

Prior to any study procedures, all subjects must document their consent for study participation and authorization for use and disclosure of health information by signing the IRB-approved Informed Consent Form. As part of the consent process, the subject will have the opportunity to ask questions of, and receive answers from the personnel conducting the study.

The investigator will notify the Study Management Center and the IRB within 5 working days if device use occurs without subject informed consent.

6 **Study Assessments and Data Management**

6.1 **Screening**

- Identify Potential Study Subjects. Refer to the Inclusion and Exclusion Criteria sections of this protocol for a complete list of eligibility criteria.

- Obtain Written Informed Consent. Each potential study participant must be given time to review the IRB-approved informed consent form, have his/her questions answered to their satisfaction and sign the form prior to any study procedures being performed. A subject will be given a copy of the informed consent form.

- Review Inclusion/Exclusion Criteria. The investigator and/or designee will review all criteria to determine if the subject is eligible for enrollment. Eligibility of all subjects must personally be confirmed by the Investigator and will be documented on the CRF.

6.2 **Enrollment**

- Assign Identification Number to Eligible Subjects. See Protocol section 6.3.

- Record Demographics, Antihypertensive Medical History and current Blood Pressure. Data will be documented in the source document and recorded on the CRF, including but not limited to the following:
  - Age
6.3 Specimen Collection

10 Collect Buccal Specimen.

Using the collection kit consisting of two buccal swabs and two uniquely barcoded tubes the investigator or designee will remove the first buccal brush and scrape the brush end across a Subject's right cheek repeatedly (for five seconds). The investigator/designee will place the brush end over the open buffer vial and press the opposite end of the swab stick to release the brush into the buffer and then close the vial. The process can be repeated on the left cheek. Each of the right and left cheek vial numbers must be recorded on the CRF and accountability log as right (R), or left (L).

15 • Adverse Event Recording
• Perform Product Accountability

6.4 Subject Numbering

Subjects meeting the criteria for enrollment (and their specimens) can be identified by unique numbers that can be assigned sequentially by order of enrollment. The pre-assigned investigational site number can be prefixed to the identification number and separated by a hyphen (e.g., site 01 would number their subjects sequentially as 01-001, 01-002, 01-003, etc.). Throughout the descriptions within the protocol the A swab will be referring to the swab that has originated from the right cheek, while the B swab will be that that has originated from the left cheek. To further clarify. Subject 01-001 can be given two barcoded tubes. These barcode numbers can be recorded for each patient. These can also be recorded as originating from the right cheek (A) or left cheek (B).

At no time should any study paperwork or specimens be marked with the subject's name or any other traceable identifier except for the informed consent form, which is signed by the subject and kept at the site. At no time should the
original (signed) or a copy of this form be collected by the Sponsor or its representative.

6.5 **Subject Completion and Withdrawal**

Once subjects undergo the sampling procedure, their study participation is complete. There are no follow-up visits. Subjects will be instructed to notify the Investigator if they experience any symptoms or complications from the sampling procedure.

Subjects are free to withdraw consent and discontinue participation in the study at any time. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator or Geneticure. The following may be justifiable reasons for the Investigator or Geneticure to remove a subject from the study:

- The subject was erroneously included in the study or was found to have an exclusion criterion.
- The subject was uncooperative.
- The subject experienced an AE/SAE during the sample collection procedure that is considered intolerable by the subject or Investigator.

To the extent possible, safety data will be collected on subjects who discontinue participation in the study due to safety reasons.

The following may be justifiable reasons for the Investigator or Geneticure to remove a specimen from the study:

- Sample is determined to be of poor or inadequate quality for analysis (e.g., contamination, insufficient material for analysis).
- The sample was erroneously included in the study.
- The specimen was not collected or processed per protocol procedures.

6.6 **Concomitant Medications/Treatment/Procedures**

This study protocol does not require change to any existing treatments or those prescribed during the course of the study by the Investigator or any other provider whom the subject sees for any medical reason. Outside of eligibility screening, there are no clinical evaluations as part of this study.
6.7  Data Management
The Investigator is responsible to ensure the accuracy, completeness, and timeliness of reported data.

All data will be sent to Geneticure who will enter it into the study database using a secure, protected Excel spreadsheet. The database will be validated prior to use in the study. All required data will be recorded on CRFs or paper facsimiles. Data collected within the CRFs will be supported by source documents as appropriate and may be updated to reflect the latest observations on the subjects participating in the study. Corrections to the source documentation can be made in a manner that does not obscure the original entry and will be dated and initialed by the Investigator or assigned designee. It is important for data entry to occur in a timely manner, therefore, data collected on source documents should be transferred into CRFs as soon as possible following study visits.

Study subject data can be reviewed at the investigational site by monitors at regular intervals throughout the study. Information on the CRFs can be compared to information originally recorded on source documents related to the study (i.e. professional notes, study-specific worksheets, etc.)

7  Genomic Core Laboratory
The subjects’ cheek vials will be sent to the Geneticure processing center. The vials will then be batched and sent to the Genomic Core laboratory for DNA extraction and genetic analysis. Following analysis, results will be sent to Geneticure for statistical analysis and DNA will be destroyed.

A protocol for the extraction and analysis will be followed to ensure consistency and objectivity.

8  Adverse Events
The procedures outlined in this protocol do not involve significant risk to subject safety. Subjects will be provided the investigator's contact information and will be instructed to notify the investigator of any adverse events they experience during or secondary to the sample collection procedures.
8.1 Definitions

8.1.1 Adverse Event
For the purposes of this study, an adverse event is defined as any undesirable/unusual medical experience that occurs to a subject in conjunction with the use of the product, whether or not considered product related, including (but not limited to) those events that result from the use as stipulated in the protocol.

The following adverse events will not be collected in this study:

- Adverse events which, in the opinion of the Investigator, are unrelated to the swab collection procedure, but rather related to the subject's underlying medical conditions or status
- Adverse events that may be related to the sample collection procedure but result only in local, mild and transient discomforts.

The Investigator is responsible for documenting all Adverse Events on the Adverse Event CRF, except for those events noted above.

8.1.2 Serious Adverse Event

A Serious adverse event is an adverse event that:

- led to death,
- led to serious deterioration in the health of a subject that
  - resulted in a life-threatening illness or injury,
  - resulted in permanent impairment of a body structure or body function,
  - required inpatient hospitalization or prolongation of existing hospitalization,
  - resulted in medical or surgical intervention to prevent permanent impairment to a body structure or a body function,
- led to fetal distress, fetal death or a congenital anomaly or birth defect.

8.2 Event Reporting

Any AE, or SAE experienced by a subject after signing the informed consent until twenty-four (24) hours following study completion or termination will be recorded in the progress notes and on the CRF. The Investigator and/or designee will continue to monitor the subject with additional assessments until the AE is considered resolved, stabilized, or is lost to follow up.
A full description of an adverse event, including the nature, date and time of onset and resolution, determination of seriousness, frequency, severity, treatment, outcome, and relationship to the study will be recorded on the Adverse Event CRF.

5 SAEs must be reported to RCRI within 48 hours of the Investigator's first knowledge of the event.

9 Statistical Methods

Following is a summary of the Statistical Analysis Plan for the study.

The following objectives have been prospectively defined; however, due to the nature of these data, additional analyses may be conducted or additional subsets may be identified that are not listed in this protocol.

9.1 Sample Size

Up to 300 subjects may be enrolled at each site. The minimum number of subjects for meaningful statistical analysis is 100 subjects.

9.2 Data Analysis

All data will be coded for statistical analysis (i.e. drug classes and genotypes will be coded numerically). All data will be analyzed with SPSS v.20. Normality of the data will be assessed using Levene's test prior to statistical analysis and any correction for non-normal data distribution will be used. Descriptive statistics will be computed (average time for blood pressure control, average number of visits to the clinician for blood pressure control, age, height, weight, BMI, etc.).

Data will be initially analysed following the collection of samples/data from 100 subjects. This will allow for direction for power calculations/etc. for future statistical analysis. Although some of the genes have been analysed individually, no mean or standard deviation data exists to allow for a true a priori power calculation. Data will be analysed again after two months or following 300 subjects for which data has been collected. Statistical tests will be corrected for the number of tests run (preservation of alpha).

Ordinary least squares regression via univariate modelling will be used to estimate the magnitude of linearity between drug class that yielded the best blood pressure control and genetic profile of the subject. Multiple regression analysis will be performed to determine the impact of confounding variables (height, weight, age, race) on blood pressure control. For all statistical analyses an alpha level of 0.05 will be used to determine statistical significance.

9.3 Other Statistical Considerations

Justification of Pooling Data across Centers
There is no need to keep the data from different centers separate for data collection. Primary reasons for not pooling blood pressure data from different centers could include different races (which we are collecting as a demographic and analyzing as a co-variate in a multiple-regression) and different cultures (i.e. southern vs. northern habits of diet, exercise, etc.). The study will take race, height, weight, age into account as co-variates in a multiple regression model, but will not be powered to take into account possible geographic influences on the data.

**Missing Data**

All patients with available data will be included in the analyses of primary and secondary objectives. Because some of the data was not recorded as part of a prospective protocol, an unknown amount of data will be permanently missing. No patients will be contacted to retrieve missing data, and no sensitivity analyses will be performed on missing data.

10 **Risk Analysis**

10.1 **Device Description**

The procedures outlined in this protocol do not involve significant risk to subject safety. Subjects will be provided the investigator's contact information and will be instructed to notify the investigator of any adverse events they experience during or secondary to the specimen collection procedures.

The collection kit includes a small, soft, brush for cheek swabbing and a buffer solution in a small vial, one of each for each cheek, two in total. Once the swab has been placed into the lysis buffer the cells are no longer viable and therefore samples are now considered to be nucleic acids and safe to be shipped via standard mail.

11 **Study Materials**

11.1 **Handling and Storage**

The Investigator must ensure that the investigational product is stored in a controlled location with limited access.

11.2 **Product Accountability**

The investigator is responsible for investigational product accountability, reconciliation and record maintenance. The investigator must maintain
investigational product accountability records throughout the course of the study.

Upon completion or termination of the study, all unused product, together with a copy of the product accountability form will be returned to Geneticure or its representative.

All supplies are to be used only for this protocol and not for any other purpose.

12 Study Administration

12.1 Subject Confidentiality

All information and data sent to Geneticure, and/or its designees concerning subjects and their participation in this study are considered confidential by Geneticure and it designees (subcontractors or contract research organization). Only authorized Geneticure personnel or approved contracted agents of Geneticure will have access to some portions of these confidential files and will act in accordance with applicable regulations as required by HIPAA. The IRBs and FDA also have the right to inspect and copy all records pertinent to this study. All data used in the reporting of the study will eliminate identifiable reference to the subjects.

12.2 Investigational Center Qualification

Investigational Center qualification visits or phone calls will be conducted by the Study Management Center prior to acceptance of the site into this study. The site qualification visit will be scheduled to include time with the Principal Investigator and other study personnel as applicable. Areas of discussion include a review of personnel training, investigator qualifications, adequacy of potential subject pool, FDA-regulated study experience, this study's specific requirements for procedures, and a review of staffing availability and appropriateness. A written report of the qualification visit will be drafted by the Study Management Center.

12.3 Site Training

Study-specific training of study personnel is the responsibility of the Sponsor or Study Management Center and the Principal Investigator. Study training will occur before the first device use. To ensure protocol and regulatory compliance as well as accurate data collection, site training will include a
detailed review of the protocol, CRF completion, study specific procedures, monitoring logistics, and regulatory requirements.

12.4 Investigator Responsibilities

The investigator is responsible for ensuring that the study is conducted according to the investigational plan and all applicable FDA regulations, including reporting and record-keeping requirements, and controlling the devices undergoing investigation and HIPAA. In addition, the principal investigator is responsible for ensuring that informed consent is obtained from each subject prior to participating in the study, as well as protecting the rights, safety and welfare of participating subjects. Specific responsibilities are listed in this investigational plan.

Records and reports must remain on file at the investigational site for a minimum of two years after the later of either the completion/termination of the investigational study or the date it is determined the records are no longer required to support submissions to regulatory authorities. They may be discarded only upon approval from Geneticure. The Principal Investigator must contact Geneticure before destroying any records and reports pertaining to the study to ensure that they no longer need to be retained. In addition, Geneticure must be contacted if the investigator plans to leave the investigational site to ensure that arrangements for a new investigator or records transfer are made prior to investigator departure.

12.4.1 Records

Records to be maintained by the investigator in the designated investigational center's study file include:

- Investigational plan and all amendments
- Signed Financial Disclosure
- IRB approval letter including consent and HIPAA authorization form(s)
- IRB Membership list or Letter of Assurance
- All correspondence relating to the study between the site and Geneticure, and the Study Management Center
- CVs and professional licenses for all investigators
- Site personnel signature and responsibility list
- Clinical monitor sign-in log
• Blank set of each version of CRFs
• Subject Screening/Enrollment log
• Investigational device accountability log including: date, quantity, lot numbers of all devices, identification of all persons the device was used on and final disposition.

The following records are maintained for each subject enrolled in the study:

• Signed Consent Form and Authorization for the Use and Disclosure of Health Information
• Complete, accurate and current CRFs and DCFs
• Adverse event reports and any supporting documentation
• Protocol deviations
• Complete medical records, including procedure reports, lab reports, professional notes, etc.

Geneticure reserves the right to secure data clarification and additional medical documentation on subjects enrolled in this study at any time.

13 Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>DCF</td>
<td>Data Clarification Form</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act of 1996</td>
</tr>
<tr>
<td>IRB/IEC</td>
<td>Institutional Review Board/Independent Ethics Committee</td>
</tr>
<tr>
<td>ITT</td>
<td>Intent-to-Treat</td>
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<tr>
<td>PP</td>
<td>Per Protocol</td>
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<td>SAE</td>
<td>Serious Adverse Event</td>
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<tr>
<td>UADE</td>
<td>Unanticipated Adverse Device Effect</td>
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Example 7: Results and Summary of Phase I Clinical Study

Introduction:

For this phase-I research study 14 genes within the Geneticure blood pressure (BP) panel were assessed as they relate to time to BP control and absolute BP values in 99 patients with hypertension. The study design utilized a post-hoc patient chart review carried out by two clinical sites through the direction of RCRI (a third-party clinical research firm) exploring genes important in drug metabolism, renal Na\(^+\) handling, vascular function, and cardiac
output (all of which can result differences in BP and response to BP therapy).

Although the primary aim was BP control in response to therapy relative to genetic data, the time on average, it takes patients to achieve BP control without consideration of genetic information was also determined.

In summary, the study demonstrated that the genes in the Geneticure panel were predictive of time to BP control in patients with hypertension. In addition, there was an effect of several of the genes being predictive of BP taken within the clinic at the time of the research study. In addition, mechanistic data was gathered for the genes that encode the alpha subunit of the epithelial Na⁺-channel (SCNN1A, rs# 2228576) and found that SCNN1A was predictive of urinary Na⁺ concentration and mean arterial BP.

**Methods:**

The BP history for patients was collected and the current BP levels were measured in patients with controlled hypertension. DNA was collected using a buccal swab and analyzed the genes within the Geneticure panel. The study sought to determine if patients with "functional" genotypes of proteins important in certain drug classes responded better if they were taking the drug that would inhibit the activity of that protein. As an example, the beta-1 adrenergic receptor (ADRB1) is important in heart rate control and patients who are on a beta-blocker can demonstrate a drop in BP because of inhibition of this protein. Therefore, one would hypothesize that if a patient with a functional protein of the ADRB1 is put on a beta-blocker early, they will demonstrate better BP control (because of a greater drop in heart rate and, therefore BP). This was assessed according to 14 genes and 3 major classes of BP drugs (diuretic, vasodilator, beta-blocker) and one drug metabolizing enzyme (CYP2D6).

**Results: Subject Characteristics**

**Demographics (n=99)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>mean±SEM</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
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</tr>
<tr>
<td>Sex (% female)</td>
<td>46</td>
</tr>
<tr>
<td>Diabetes (% with)</td>
<td>28±4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86±1.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169±1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.9±0.4</td>
</tr>
</tbody>
</table>
Results: Blood Pressure Data (n=99)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial SBP (mm Hg)</td>
<td>151±2</td>
</tr>
<tr>
<td>Initial DBP (mmHg)</td>
<td>91±1</td>
</tr>
<tr>
<td>Initial MAP (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Lowest SBP in past two years (mmHg)</td>
<td>115±1</td>
</tr>
<tr>
<td>Lowest DBP in past two years (mmHg)</td>
<td>72±1</td>
</tr>
<tr>
<td>Current SBP (mmHg)</td>
<td>134±2</td>
</tr>
<tr>
<td>Current DBP (mm Hg)</td>
<td>82±1</td>
</tr>
<tr>
<td>Current MAP (mmHg)</td>
<td>99±1</td>
</tr>
<tr>
<td>Time to BP control (months)</td>
<td>22±10</td>
</tr>
<tr>
<td>Clinic Visits in the Past two years for HTN</td>
<td>3.610.3</td>
</tr>
</tbody>
</table>

5 Results: Current Blood Pressure Therapy Information

Drug Class Usage (n=99)

<table>
<thead>
<tr>
<th>Variable</th>
<th>mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Classes of Drugs for HTN</td>
<td>1.8±0.08</td>
</tr>
<tr>
<td>Diuretic (% taking)</td>
<td>42±5</td>
</tr>
<tr>
<td>ACE Inhibitor (% taking)</td>
<td>62±5</td>
</tr>
<tr>
<td>ARB (% taking)</td>
<td>27±5</td>
</tr>
<tr>
<td>B-Blocker (% taking)</td>
<td>33±5</td>
</tr>
<tr>
<td>Ca⁺ Channel Blocker (% taking)</td>
<td>16±4</td>
</tr>
</tbody>
</table>

These data describe the number of different drug classes that the patients were taking. In addition, we assessed the percent of subjects who were on drugs within the vasodilator class (ACE-inhibitor and ARB), the cardiac class (B-blocker Ca⁺ channel blocker), and the renal class (diuretic).
Time to Control According to Drug Class (n=99)

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Months for Control</th>
<th>Clinic Visits/Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On the Drug Class</td>
<td>Not on the Drug Class</td>
</tr>
<tr>
<td>Diuretic</td>
<td>39.5±20.4</td>
<td>7.9±4.2</td>
</tr>
<tr>
<td>ACE Inhibitor</td>
<td>22.3±11.4</td>
<td>22.5±16.4</td>
</tr>
<tr>
<td>Angiotensin Receptor Blocker</td>
<td>32.8±23.1</td>
<td>17.1±9.1</td>
</tr>
<tr>
<td>Beta Blocker</td>
<td>24.5±16.9</td>
<td>21.2±12.0</td>
</tr>
<tr>
<td>Car Channel Blocker</td>
<td>9.9±4.5</td>
<td>25.8±11.7</td>
</tr>
</tbody>
</table>

*p<0.05 compared to those patients who were on the class of drugs
These data describe the time it took for BP control according to which class of drugs the patient was taking. While there are no significant differences in months taken for BP control according to drug class, there was an effect of number of clinic visits (specific to hypertension) within the past 2 years according to drug class. Patients using beta-blockade and diuretic therapy to control their BP had fewer clinic visits, when compared to those patients not on these therapies. Patients on an ACE-inhibitor had significantly more clinic visits per year, when compared to patients not on this therapy.

**Blood Pressure Control According to Genotypes (n=86)**

1. *Genes important in renal Na⁺ handling (and those that are differentially responsive to diuretic therapy).*

<table>
<thead>
<tr>
<th><strong>WNK1 (RS# 1159744)</strong></th>
<th>On Target Therapy (Diuretic)</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td>GG</td>
<td>C·containing</td>
<td>GG</td>
</tr>
<tr>
<td>n</td>
<td>26</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>133.7±3.2</td>
<td>133.5±2.3</td>
<td>137.8±4.1</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>79.7±2.3</td>
<td>84.6±2.0</td>
<td>88.7±2.7*</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mm Hg)</td>
<td>97.7±2.4</td>
<td>100.9±1.81</td>
<td>105.1±2.7*</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>3.6±1.4</td>
<td>4.8±2.6</td>
<td>8.2±5.6</td>
</tr>
</tbody>
</table>

*P<0.05 compared to same genotype not on target therapy.

<table>
<thead>
<tr>
<th><strong>SLC12A3 (RS# 1529927)</strong></th>
<th>On Target Therapy (Diuretic)</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td>GG</td>
<td>C·containing</td>
<td>GG</td>
</tr>
<tr>
<td>n</td>
<td>45</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>134.2±2.31</td>
<td>136.8±5.9</td>
<td>136.4±3.4</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>81.8±1.7</td>
<td>86.5±2.3</td>
<td>85.9±2.5</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mm Hg)</td>
<td>99.3±1.7</td>
<td>103.3±3.1</td>
<td>102.7±2.4</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>2.5±0.7</td>
<td>17.7±7</td>
<td>10.5±3.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>WNK1 (RS# 2107614)</strong></th>
<th>On Target Therapy</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>GG</td>
<td>C-containing</td>
<td>G</td>
</tr>
<tr>
<td>------------</td>
<td>-----</td>
<td>--------------</td>
<td>----</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>41</td>
<td>7</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>130.3±5.6</td>
<td>134.4±2.1</td>
<td>147.7±7.9</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>80.4±4.7</td>
<td>82.4±1.6</td>
<td>84.6±4</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mm Hg)</td>
<td>97.1±4.6</td>
<td>99.7±1.6</td>
<td>106±4.4</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>10.5±7.2</td>
<td>2.6±0.9</td>
<td>10.5±9.5</td>
</tr>
</tbody>
</table>

### Alpha Adducin (RS# 4961)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>134.2±2.3</td>
<td>135.0±5.3</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>83.1±1.8</td>
<td>79.7±3.3</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mm Hg)</td>
<td>100.2±1.8</td>
<td>98.2±3.6</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>4.4±1.6</td>
<td>3.6±15.1</td>
</tr>
</tbody>
</table>

Of the four genes explored in the clinical study RS# 1159744 (the gene that encodes cytoplasmic serine-threonine kinase that is expressed in the kidney, WNK-1) was most predictive of response to therapy. Patients with the C genotype of WNK-1 had the best response to therapy demonstrating 8mmHg lower DBP, when compared to patients with this genotype who were not on diuretic therapy. Subjects who were homozygous for G for this gene actually had a lower blood pressure if they were not on a diuretic, indicating that they may be benefiting from alternate therapy. Although just a trend (due to small sample size of the minor allele) the C polymorphism of SLC12A3 also may be predictive of response to diuretic therapy with patients demonstrating an 11mmHg drop in DBP with therapy, compared to the G polymorphism which demonstrated a small increase in DBP with therapy.

In addition to this clinical BP data 24-hour urinary and resting BP data were gathered according to genetic variation of the alpha sub-unit of the epithelial Na⁺ channel (SCNN1A, RS# 2228576). It was found that subjects homozygous for the T variant of SCNN1A demonstrated more Na⁺ excretion.
from the kidneys and they also demonstrated lower mean arterial blood pressure, when compared to genotype groups containing the A variant (See FIGs. 8 and 9).

2. Genes important in cardiac function (and those that may respond differentially to beta-blocker therapy).

5 The beta-1 adrenergic receptor (ADRB1) is important in controlling heart rate and cardiac contractility.

<table>
<thead>
<tr>
<th>Beta-1 Adrenergic Receptor 49 (RS# 1801252)</th>
<th>No</th>
<th>Thr-containing</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Ser/Ser</td>
<td>Thr-containing</td>
<td>Ser/Ser</td>
</tr>
<tr>
<td>n</td>
<td>1</td>
<td>55</td>
<td>2</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>137</td>
<td>136.4±2.3</td>
<td>136±6</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>86</td>
<td>85.5±1.7</td>
<td>75±3</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mm Hg)</td>
<td>103</td>
<td>102.4±1.7</td>
<td>95±0</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>N/A</td>
<td>7.0±2.4</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*P<0.05 compared to same genotype not on target therapy.

<table>
<thead>
<tr>
<th>Beta-1 Adrenergic Receptor 389 (RS# 1801253)</th>
<th>No</th>
<th>C-containing</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>GG</td>
<td>C-containing</td>
<td>GG</td>
</tr>
<tr>
<td>n</td>
<td>56</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>136.4±2.3</td>
<td>132.6±3.0</td>
<td></td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>85.5±1.7</td>
<td>80.0±2.0*</td>
<td></td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mm Hg)</td>
<td>102.4±1.7</td>
<td>97.5±1.98</td>
<td></td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>7.0±2.3</td>
<td>8.8±3.5</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 compared to same genotype not on target therapy.

These data indicate a differential BP response to beta-blocker therapy according to genetic variation at position 49 of the ADRB1. Specifically, the inventors found that subjects with the Ser genotype at position 49 of ADRB1 benefit from beta-blocker therapy with an average drop in DBP of 11mmHg, compared with a drop of 5mmHg with Thr at this position. Therefore, although patients with the Thr polymorphism also demonstrated a drop in BP with beta-blocker therapy, the effect was most pronounced in patients with the Ser polymorphism.
The beta-2 adrenergic receptor (ADRB2) is important in cardiac contractility, which controls stroke volume, and can influence BP through differences in cardiac output.

<table>
<thead>
<tr>
<th>Beta-2 Adrenergic Receptor 16 (RS# 1042713)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>On Target Therapy (B-Blocker)</strong></td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>Months to BP Control</td>
</tr>
</tbody>
</table>

*P<0.05 compared to same genotype not on target therapy

<table>
<thead>
<tr>
<th>Beta-2 Adrenergic Receptor 27 (RS# 1042714)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>On Target Therapy (B-Blocker)</strong></td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>Months to BP Control</td>
</tr>
</tbody>
</table>

These data demonstrate a generally favorable response to beta-blocker therapy with both genotype groups. However, the Gly16 genotype demonstrated a statistically significant difference in BP control if the patients were on a beta-blocker (drop in DBP of 5mmHg), when compared to patients with the Arg16 genotype. Generally, there is a similar pattern for a greater drop in BP with subjects who have the most functional gene that encodes the ADRB2 (Gly at position 16 and Glu at position 27). There is strong linkage disequilibrium between these two sites (amino acids 16 and 27), so the similar response between the sites is expected.

Observations on Genetic variation of cytochrome P450 2D6 (CYP2D6), which is important in drug metabolism, especially of particular beta-blockers.
<table>
<thead>
<tr>
<th>CYP 2D6 (RS#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On Target Therapy (B-Blocker)</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>CC</td>
</tr>
<tr>
<td>T-Containing</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>23</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>140.6±2.7</td>
</tr>
<tr>
<td>128.5±3.1</td>
</tr>
<tr>
<td>i133.4±3.6</td>
</tr>
<tr>
<td>130.0±5.7</td>
</tr>
<tr>
<td>iDiastolic Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>86.0±2.4</td>
</tr>
<tr>
<td>83.2±2.4</td>
</tr>
<tr>
<td>i79.2±2.1*</td>
</tr>
<tr>
<td>82.6±5.3</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>104.2±2.3</td>
</tr>
<tr>
<td>98.3±2.4</td>
</tr>
<tr>
<td>i97.3±2.2*</td>
</tr>
<tr>
<td>98.4±4.8</td>
</tr>
<tr>
<td>iMonths to BP Control</td>
</tr>
<tr>
<td>7.8±2.9</td>
</tr>
<tr>
<td>5.08±3.7</td>
</tr>
<tr>
<td>6.25±3.2</td>
</tr>
<tr>
<td>16.5±10.8</td>
</tr>
</tbody>
</table>

i*P<0.05 compared to same genotype not on target therapy

These data demonstrate that the CC homozygous group of CYP2D6 demonstrates the greatest response to beta-blocker therapy, when compared to the CT and TT groups. Patients with the CC polymorphism had demonstrated a 6mmHg lower DBP and a 7mmHg lower MAP when on beta-blocker therapy, compared to this genotype not on beta-blocker therapy. In contrast, patients in the T-containing group (those with the CT and TT genotypes) did not respond to beta-blocker therapy.

3. Genes important in vascular function (and those that may respond differentially to vasodilator therapy).

The following are observations on the genetic variation of the angiotensin gene (encoding a precursor to angiotensin-II, a potent vasoconstrictor, which is converted via angiotensin converting enzyme, ACE) and the responses to various therapies.
<table>
<thead>
<tr>
<th>Angiotensin (RS# 699)</th>
<th>On Target Therapy (Angiotensin Receptor Blocker)</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>CC</td>
<td>T-containing</td>
<td>CC</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>135.3±2.6</td>
<td>135.1±2.6</td>
<td>135.0±7.4</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>89.5±2.9</td>
<td>82.6±1.7</td>
<td>77.5±7.8</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
<td>104.7±2.6</td>
<td>100.1±1.7</td>
<td>96.6±7.5</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>2.7±1.3</td>
<td>6.7±2.7</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Angiotensin (RS# 699)</th>
<th>On Target Therapy (ACE-Inhibitor)</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>CC</td>
<td>T-containing</td>
<td>CC</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>131.7±5.3</td>
<td>133.7±3.5</td>
<td>136.8±2.7</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>78.5±5.0</td>
<td>80.4±2.1</td>
<td>90.8±3.2*</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
<td>96.2±4.7</td>
<td>98.2±*</td>
<td>106.2±2.8*</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>6.7±3.1</td>
<td>7.7±3.2</td>
<td>2.3±1.7</td>
</tr>
</tbody>
</table>

*P<0.05 compared to same genotype not on target therapy

<table>
<thead>
<tr>
<th>All Receptor Type-1 (RS# 5186)</th>
<th>On Target Therapy (Angiotensin Receptor Blocker)</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>AA</td>
<td>C-containing</td>
<td>AA</td>
</tr>
<tr>
<td>n</td>
<td>39</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>137.3±2.6</td>
<td>131.0±3.1</td>
<td>136.3±4.6</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>88.4±2.1</td>
<td>80.9±2.0</td>
<td>87.6±3.9</td>
</tr>
<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
<td>102.7±2.0</td>
<td>97.6±2.1</td>
<td>103.8±2.9</td>
</tr>
<tr>
<td>Months to BP Control</td>
<td>7.3±2.9</td>
<td>3.8±2.5</td>
<td>13.4±7.8</td>
</tr>
</tbody>
</table>
These data indicate that patients homozygous for the C genotype of angiotensin may benefit from an angiotensin receptor blocker (ARB). When on an ARB, patients with the CC genotype demonstrated a 12 mmHg lower DBP when compared to patients with this genotype who were not on this therapy. In contrast, patients in the T-containing group (those with the CT or TT genotypes) did not show a response to ARB therapy. Furthermore, inhibition of ACE (which converts angiotensin-I to angiotensin-II) results in higher BP levels, possibly due to a "build-up" of angiotensin. Therefore, these data indicate that patients homozygous for C should be given an angiotensin receptor blocker with an ACE inhibitor.

Angiotensin converting enzyme (ACE) genotype and ACE-inhibition

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No</th>
<th>Del·Containing</th>
<th>Yes</th>
<th>Del·Containing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACE (RS# 1799752)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>On Target Therapy (ACE-Inhibition)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ins/Ins</strong></td>
<td>3</td>
<td>27</td>
<td>12</td>
<td>47</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure (mmHg)</strong></td>
<td>142.7±11.1</td>
<td>132.3±3.1</td>
<td>129.5±3.5</td>
<td>137.1±2.6</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure (mm Hg)</strong></td>
<td>79.6±6.1</td>
<td>80.0±2.1</td>
<td>80.4±4.4</td>
<td>85.7±1.9*</td>
</tr>
<tr>
<td><strong>Mean Arterial Blood Pressure (mm Hg)</strong></td>
<td>100.7±7.7</td>
<td>97.4±2.1</td>
<td>96.8±3.8</td>
<td>102.8±1.8</td>
</tr>
<tr>
<td><strong>Months to BP Control</strong></td>
<td>4.7±3.7</td>
<td>8.0±3</td>
<td>7.4±5.8</td>
<td>7.3±3.0</td>
</tr>
</tbody>
</table>

*P<0.05 compared to same genotype not on target therapy.

These data indicate that those with the Insertion polymorphism (Ins) of the ACE gene will respond best to ACE-inhibition. Patients with the Del polymorphism actually demonstrated higher DBP with ACE-inhibition, when compared to this patient group not on ACE-inhibitors.

Observations on Renin Genotype and Angiotensin Receptor Blocker

Renin is a precursor to angiotensin and, therefore, patients with a functional genotype of renin may benefit from Angiotensin Receptor Blocker (ARB) therapy because a more functional genotype can lead to greater angiotensin levels which can result in high BP.
These data indicate that the functional genotype of renin \((A)\) may benefit from ARB therapy. Specifically, the BP response to therapy was not significant, however, the response to therapy time was pronounced. Patients who have the functional genotype of renin (the AG and AA genotype groups) demonstrate a much shorter time to BP control, when compared to patients within this group who do not take this therapy. In contrast, patients in the GG group demonstrate a longer time to control if they take this therapy, possibly due to greater response to another class of drugs.

**References:**


All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby specifically incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

The specific methods, devices, and kits described herein are representative of preferred embodiments and are exemplary and not intended as
limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and the methods and processes are not necessarily restricted to the orders of steps indicated herein or in the claims.

As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

Thus, for example, a reference to "a nucleic acid" or "a polypeptide" includes a plurality of such nucleic acids or polypeptides (for example, a solution of nucleic acids or polypeptides or a series of nucleic acid or polypeptide preparations), and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims and statements of the invention.
WHAT IS CLAIMED:

1. A kit comprising: binding entities or probes for detecting 2-14 different polypeptide or nucleic acid segments, respectively, wherein each of the binding entities and/or probes selectively binds to one of the following targets:

   a) ADRBI as a target:
      a. wherein the ADRBI polypeptide has a serine or glycine at position 49;
      b. wherein the ADRBI polypeptide has an arginine or glycine at position 389;
      c. wherein the ADRBI nucleic acid comprises rs1801252 (SEQ ID NO:2) with an adenine or guanine at the rs1801252 variable position; and/or
      d. wherein the ADRBI nucleic acid comprises rs1801253 (SEQ ID NO:3) with a cytosine or guanine at the rs1801253 variable position;

   b) ADRB2 as a target:
      1. wherein the ADRB2 (β2AR) polypeptide has a glycine or arginine at position 16;
      2. wherein the ADRB2 (β2AR) polypeptide has glutamic acid or glutamine at position 27;
      3. wherein the ADRB2 nucleic acid comprises rs1042713 (SEQ ID NO:6) with or an adenine or a guanine at the rs1042713 variable position; and/or
      4. wherein the ADRB2 nucleic acid comprises rs1042714 (SEQ ID NO:7) with a cytosine or a guanine at the rs1042714 variable position;

   c) CYP2D6 as a target:
      a. wherein the CYP2D6 nucleic acid encodes a CYP2D6-defective gene product; and/or
      b. wherein the CYP2D6 nucleic acid comprises rs3892097 (SEQ ID NO:10) with an adenine or a guanine at the rs3892097 variable position;
d) angiotensin (AGT) as a target:
   1. wherein the angiotensin polypeptide has methionine or threonine at position 268; and/or
   2. wherein the angiotensin (AGT) nucleic acid comprises rs699 (SEQ ID NO:14) with cytosine or thymine at the AGT variable position;

e) angiotensin receptor type-I (AGTIR) as a target, wherein the angiotensin receptor type-I (AGTIR) nucleic acid comprises rs5186 (SEQ ID NO:16) at least partially in an AGTIR intron, with an adenine or a cytosine at the rs5186 variable position;

f) renin as a target, wherein the renin nucleic acid comprises rs12750834 (SEQ ID NO:19) with an adenine or a guanine at the rs12750834 variable position;

g) Na+ channel (SCNN1A) as a target:
   1. wherein the Na+ channel (SCNN1A) polypeptide has threonine or alanine at about position 663 or at about position 722; and/or
   2. wherein the Na+ channel (SCNN1A) nucleic acid comprises rs2228576 (SEQ ID NO:22) with an adenine or a guanine at the rs2228576 variable position;

h) alpha adducin as a target:
   1. wherein the alpha adducin polypeptide has glycine or tryptophan at position 460; and/or
   2. wherein the alpha adducin (ADD1) nucleic acid comprises rs4961 (SEQ ID NO:27) with a guanine or thymine at the rs4961 variable position;

i) sodium (Na+) chloride (Cl-) co-transporter (SLC12A3) as a target:
   1. wherein the sodium (Na+) chloride (Cl-) co-transporter (SLC12A3) polypeptide with glycine or alanine at about position 264; and/or
   2. wherein the sodium (Na+) chloride (Cl-) co-transporter (SLC12A3) nucleic acid comprises rs1529927 (SEQ ID NO:30) with a cytosine or guanine at the rs1529927 variable position 264; and/or
j) WNK1 as a target:

1. wherein the WNK1 nucleic acid comprises rs2107614 (SEQ ID NO:33) with a cytosine or thymine at the rs2107614 variable position;

2. wherein the WNK1 nucleic acid comprises rs159744 (SEQ ID NO:34) with a cytosine or guanine at the rs159744 variable position; and

instructions for performing an assay to determine whether the test sample comprises any of the polypeptide or the nucleic acid target segments.

2. The kit of claim 1, comprising binding entities or probes for detecting 3-46, 4-46, 5-46, 6-46, 7-46, 8-46, 9-46, 10-46 different polypeptides or nucleic acid segments.


4. The kit of any one of claims 1-3, comprising binding entities for detecting 3-18, 4-18, 5-18, 6-18, 3-18, 7-18, 8-18, 9-18, 10-18, 11-18, 12-18, 13-18, 14-18, 15-18, 16-18, 17-18, 18-18, 19-18, 20-18, 21-18, 22-18, 23-18, 24-18, 25-18, or 26-18 different polypeptide segments (epitopes).

5. The kit of any one of claims 1-4, wherein one or more of the binding entities or probes are covalently linked to a distinct label.

6. The kit of any one of claims 1-5, wherein the binding entities or probes are covalently bound to a microarray, microchip, bead, assay plate, or nanochip.

7. The kit of any one of claims 1-6, wherein the instructions describe procedures for determining whether a subject from whom the sample is
obtained is heterozygous or homozygous for any of the nucleic acid targets.

8. The kit of any one of claims 1-7, wherein the instructions describe procedures for performing assays on a test sample, or components extracted from the test sample, and wherein one or more of the assays comprise single nucleotide polymorphism (SNP) analysis, polymerase chain reaction (PCR), quantitative nucleic acid amplification reactions (e.g., quantitative polymerase chain reaction), primer extension analysis, nucleic acid hybridization, nucleic acid sequencing, Northern blot analysis, immunoassay, immunosorbant assay (ELISA), radioimmunoassay (RIA), immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, immunoblotting, mass spectrometry, gel electrophoresis, or a combination thereof.

9. The kit of any one of claims 1-8, further comprising reagents for lysing cells, isolating nucleic acids, stabilizing nucleic acids, storing nucleic acids, amplifying nucleic acids, cleaving nucleic acids, protease inhibitors, nuclease inhibitors, labeling a hybridization complex formed between a probe and a target nucleic acid, labeling a complex formed between an antibody and a polypeptide target.

10. A method comprising:
   a. obtaining a test sample from a subject suspected of having high blood pressure;
   b. performing at least two assays to determine whether the test sample comprises:
      1. an ADRB1 polypeptide or an ADRB1 nucleic acid:
         a. wherein the ADRB1 polypeptide has a serine or glycine at position 49;
         b. wherein the ADRB1 polypeptide has an arginine or glycine at position 389;
         c. wherein the ADRB1 nucleic acid comprises rs1801252 (SEQ ID NO:2) with an adenine or guanine at the rs1801252 variable position; and/or
d. wherein the ADRBI nucleic acid comprises rs1801253 (SEQ ID NO:3) with a cytosine or guanine at the rs1801253 variable position;

2. an ADRB2 polypeptide or an ADRB2 nucleic acid:
   a. wherein the ADRB2 (β2AR) polypeptide has a glycine or arginine at position 16;
   b. wherein the ADRB2 (β2AR) polypeptide has glutamic acid or glutamine at position 27;
   c. wherein the ADRB2 nucleic acid comprises rs1042713 (SEQ ID NO:6) with or an adenine or a guanine at the rs1042713 variable position; and/or
   d. wherein the ADRB2 nucleic acid comprises rs1042714 (SEQ ID NO:7) with a cytosine or a guanine at the rs1042714 variable position;

3. a CYP2D6 polypeptide or a CYP2D6 nucleic acid:
   a. wherein the CYP2D6 nucleic acid encodes a CYP2D6-defective gene product; and/or
   b. wherein the CYP2D6 nucleic acid comprises rs3892097 (SEQ ID NO:10) with an adenine or a guanine at the rs3892097 variable position;

4. an angiotensin polypeptide or an angiotensin (AGT) nucleic acid:
   a. wherein the angiotensin polypeptide has methionine or threonine at position 268; and/or
   b. wherein the angiotensin (AGT) nucleic acid comprises rs699 (SEQ ID NO:14) with cytosine or thymine at the AGT variable position;

5. an angiotensin receptor type-I (AGTIR) nucleic acid comprising rs5186 (SEQ ID NO:16) at least partially in an AGTIR intron, with an adenine or a cytosine at the rs5186 variable position;

6. a renin nucleic acid comprising rs12750834 (SEQ ID NO:19) with an adenine or a guanine at the rs12750834 variable position;
7. a Na\(^+\) channel (SCNN1A) polypeptide or a Na\(^+\) channel (SCNN1A) nucleic acid:
   a. wherein the Na\(^+\) channel (SCNN1A) polypeptide has threonine or alanine at about position 663 or at about position 722; and/or
   b. wherein the Na\(^+\) channel (SCNN1A) nucleic acid comprises rs2228576 (SEQ ID NO:22) with an adenine or a guanine at the rs2228576 variable position;

8. an alpha adducin polypeptide or an alpha adducin nucleic acid;
   a. wherein the alpha adducin polypeptide has glycine or tryptophan at position 460; and/or
   b. wherein the alpha adducin (ADD1) nucleic acid comprises rs4961 (SEQ ID NO:27) with a guanine or thymine at the rs4961 variable position;

9. a sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (SLC12A3) polypeptide or a sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (SLC12A3):
   a. wherein the sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (SLC12A3) polypeptide has glycine or alanine at about position 264; and/or
   b. wherein the sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (SLC12A3) nucleic acid comprises rs1529927 (SEQ ID NO:30) with a cytosine or guanine at the rs1529927 variable position 264; and/or

10. a WNK1 polypeptide or a WNK1 nucleic acid:
    a. wherein the WNK1 nucleic acid comprises rs2107614 (SEQ ID NO:33) with a cytosine or thymine at the rs2107614 variable position; and/or
    b. wherein the WNK1 nucleic acid comprises rs159744 (SEQ ID NO:34) with a cytosine or guanine at the rs1 59744 variable position.
11. The method of claim 10, comprising performing at least three assays, or at least four assays, or at least five assays, or at least six assays on the sample to determine if at least three, or at least four, or at least five, or at least six of the nucleic acids or polypeptides are present in the sample.

12. The method of claim 10 or 11, comprising performing assays on the sample to determine if any or all of the nucleic acids or polypeptides are present in the sample.

13. The method of any one of claims 10-12, wherein the at least two assays are performed simultaneously.

14. The method of any one of claims 10-13, wherein at least three, or at least four assays, or at least five assays are performed simultaneously to determine if at least three, or at least four, or at least five of the nucleic acids or polypeptides are present in the sample.

15. The method of any one of claims 10-14, wherein all of the assays are performed simultaneously to determine if any of the nucleic acids or polypeptides is present in the sample.

16. The method of any one of claims 10-14, further comprising determining whether the subject from whom the sample is obtained is heterozygous or homozygous for any of the nucleic acids.

17. The method of any one of claims 10-16, wherein the assays are performed by microarray, microchip, assay plate, or nanochip analysis.

18. The method of any one of claims 10-17, wherein the assays are performed on a microarray, microchip, assay plate, bead, or nanochip comprising one or more nucleic acid probes, wherein each probe can separately hybridize to one of the nucleic acids under stringent or highly stringent hybridization conditions.
19. The method of any one of claims 10-18, wherein the assays are performed on a microarray, microchip, assay plate, bead, or nanochip comprising one or more antibodies, wherein each antibody separately and selectively can bind to one of the polypeptides.

20. The method of any one of claims 10-19, wherein performing the assays comprises subjecting the test sample, or components extracted from the test sample, to one or more of the assays comprising single nucleotide polymorphism (SNP) analysis, polymerase chain reaction (PCR), quantitative nucleic acid amplification reactions (e.g., quantitative polymerase chain reaction), primer extension analysis, nucleic acid hybridization, nucleic acid sequencing, Northern blot analysis, immunoassay, immunosorbant assay (ELISA), radioimmunoassay (RIA), immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, immunoblotting, mass spectrometry, gel electrophoresis, or a combination thereof.

21. The method of any one of claims 10-20, further comprising administering a beta-blocker drug to the subject if the test sample comprises:

1. an ADRB1 (PiAR) polypeptide with serine at about position 49;
2. an ADRB1 nucleic acid with rs1801252 (SEQ ID NO:2), wherein the rs1801252 variable nucleotide is adenine;
3. an ADRB1 (PiAR) polypeptide with arginine at about position 389;
4. an ADRB1 nucleic acid with rs1801253 (SEQ ID NO:3), wherein the rs1801253 variable nucleotide is cytosine;
5. an ADRB2 (β2AR) polypeptide with glycine at about position 16;
6. an ADRB2 nucleic acid with rs1042713 (SEQ ID NO:6), wherein the rs1042713 variable nucleotide is guanine;
7. an ADRB2 (β2AR) polypeptide with glutamic acid at about position 27; and/or
8. an ADRB2 nucleic acid with rs1042714 (SEQ ID NO:7), wherein the rs1042714 variable nucleotide is guanine.
22. The method of any one of claims 10-21, further comprising administering atenolol or carvedilol to the subject if the test sample comprises a CYP2D6 polypeptide or a CYP2D6 nucleic acid a CYP2D6-defective mutation.

23. The method of any one of claims 10-22, further comprising administering atenolol or carvedilol to the subject if the method shows that the patient is homozygous for a CYP2D6 nucleic acid comprising rs3892097 (SEQ ID NO: 10), wherein the rs3892097 variable nucleotide is adenine (i.e., the subject is homozygous A/A at the rs3892097 variable position).

24. The method of any one of claims 10-23, further comprising administering low doses of Metoprolol or Propranolol to the subject if the method shows that the patient is homozygous for a CYP2D6 nucleic acid comprising rs3892097 (SEQ ID NO: 10), wherein the rs3892097 variable nucleotide is adenine (i.e., the subject is homozygous A/A at the rs3892097 variable position), wherein the low doses are less than would be administered to a subject who is not A/A homozygous at the rs3892097 variable position.

25. The method of any one of claims 10-24, further comprising administering an ACE inhibitor to the subject if the test sample comprises ACE nucleic acid comprising rs1799752 (SEQ ID NO: 12) with the bracketed nucleic acid segment deleted (i.e., the rs1799752 segment has the following sequence (SEQ ID NO:35):

        TCCCAT TTCTCTAGACCTGCTGCCTATACAGTCACT TTTATGTGGT TTC G.

26. The method of any one of claims 10-25, further comprising administering an ACE inhibitor or an angiotensin receptor blocker to the subject if the test sample comprises:

   1. an angiotensin or angiotensinogen polypeptide with a threonine at about position 235 or 268; and/or
   2. an angiotensin nucleic acid with rs699 (SEQ ID NO: 14), wherein the rs699 variable nucleotide is cytosine.
27. The method of any one of claims 10-26, further comprising administering an angiotensin receptor inhibitor or blocker to the subject if the test sample comprises an angiotensin II receptor (type-I) nucleic acid with rs5186 (SEQ ID NO: 16), wherein the rs5186 variable nucleotide is a cytosine.

28. The method of any one of claims 10-27, further comprising administering an angiotensin II receptor inhibitor or blocker to the subject if the test sample comprises a renin nucleic acid with rs12750834 (SEQ ID NO: 20), wherein the rs12750834 variable position is a cytosine.

29. The method of any one of claims 10-28, further comprising administering a diuretic if the test sample comprises:
   1. a Na⁺ channel (SCNN1A) polypeptide with a threonine at about position 663 or at about position 722;
   2. a Na⁺ channel (SCNN1A) nucleic acid with rs2228576 (SEQ ID NO: 22), wherein the rs2228576 variable position is an adenine;
   3. an alpha adducin polypeptide with tryptophan at about position 460;
   4. an alpha adducin nucleic acid with rs4961 (SEQ ID NO: 27), wherein the rs4961 variable position is thymine;
   5. a sodium (Na⁺) chloride (Cl⁻) co-transporter (SLC12A3) polypeptide with an alanine at about position 264;
   6. a SLC12A3 nucleic acid with rs1529927 (SEQ ID NO: 30), wherein the rs1529927 variable position is cytosine;
   7. a WNK1 nucleic acid with rs2107614 (SEQ ID NO: 33), wherein the rs2107614 variable position is cytosine;
   8. a WNK1 nucleic acid with rs1 59744 (SEQ ID NO: 34), wherein the rs1 59744 variable position is cytosine; or
   9. any combination thereof.

30. The method of any one of claims 10-29, wherein obtaining the test comprises directly withdrawing the test sample from the subject, or indirectly receiving the test sample from a person or entity who has withdrawn the test sample from the subject.
31. A method comprising:
   a. obtaining a test sample from a subject;
   b. administering a beta-blocker drug to the subject if the test sample
      comprises:
      1. an ADRB1 (βAR1) polypeptide with serine at about position 49;
      2. an ADRB1 nucleic acid with rs1801252 (SEQ ID NO:2), wherein
         the rs1801252 variable nucleotide is adenine;
      3. an ADRB1 (PiAR) polypeptide with arginine at about position
         389;
      4. an ADRB1 nucleic acid with rs1801253 (SEQ ID NO:3), wherein
         the rs1801253 variable nucleotide is cytosine;
      5. an ADRB2 (β2AR) polypeptide with glycine at about position 16;
      6. an ADRB2 nucleic acid with rs042713 (SEQ ID NO:6), wherein
         the rs042713 variable nucleotide is guanine;
      7. an ADRB2 (β2AR) polypeptide with glutamic acid at about
         position 27; and/or
      8. an ADRB2 nucleic acid with rs042714 (SEQ ID NO:7), wherein
         the rs042714 variable nucleotide is guanine;
   c. administering atenolol or carvedilol to the subject if the test sample
      comprises a CYP2D6 polypeptide or a CYP2D6 nucleic acid with a
      CYP2D6-defective mutation;
   d. administering atenolol or carvedilol to the subject if the subject is
      homozygous for a CYP2D6 nucleic acid comprising rs3892097 (SEQ
      ID NO:10), wherein the rs3892097 variable nucleotide is adenine
      (i.e., the subject is homozygous A/A at the rs3892097 variable
      position);
   e. administering low doses of Metroprolol or Propranolol to the subject
      if the subject is homozygous for a CYP2D6 nucleic acid comprising
      rs3892097 (SEQ ID NO:10), wherein the rs3892097 variable
      nucleotide is adenine (i.e., the subject is homozygous A/A at the
      rs3892097 variable position), wherein the low doses are less than
      would be administered to a subject who is not A/A homozygous at
      the rs3892097 variable position;
f. administering an ACE inhibitor or an angiotensin receptor blocker to the subject if the test sample comprises:
   1. an angiotensin or angiotensinogen polypeptide with a threonine at about position 235 or 268; and/or
   2. an angiotensin nucleic acid with rs699 (SEQ ID NO: 14), wherein the rs699 variable nucleotide is cytosine;

g. administering an angiotensin II receptor inhibitor or blocker to the subject if the test sample comprises an angiotensin II receptor (type-I) nucleic acid with rs5186 (SEQ ID NO: 16), wherein the rs5186 variable nucleotide is a cytosine;

h. administering an angiotensin II receptor inhibitor or blocker to the subject if the test sample comprises a renin nucleic acid with rs12750834 (SEQ ID NO: 20), wherein the rs12750834 variable position is a cytosine;

i. administering a diuretic if the test sample comprises:
   1. a Na+ channel (SCNN1A) polypeptide or a Na+ channel (SCNN1A) nucleic acid encoding threonine at position 663;
   2. an adducin polypeptide or an adducin nucleic acid encoding tryptophan at position 460;
   3. a sodium (Na+) chloride (Cl-) co-transporter (SLC12A3) polypeptide or a SLC12A3 nucleic acid encoding alanine at position 264;
   4. a WNK1 nucleic acid with a cytosine detectable by SNP analysis using rs1 159744 or rs2107614; or
   5. any combination thereof.

32. The method of claim 31, further comprising performing at least two assays on the test sample to determine if at least two of the nucleic acids or polypeptides are present in the sample.

33. The method of claim 31 or 32, further comprising performing at least three assays, or at least four assays, or at least five assays, or at least six assays on the test sample, to determine if at least three, or at least four, or at least five, or at least six of the nucleic acids or polypeptides are present in the sample.
34. The method of any one of claims 31-33, comprising performing assays on the sample to determine if any or all of the nucleic acids or polypeptides are present in the sample.

35. The method of any one of claims 31-34, wherein the at least two assays are performed simultaneously.

36. The method of any one of claims 31-35, wherein at least three, or at least four assays, or at least five assays, or at least six assays are performed simultaneously to determine if at least three, or at least four, or at least five, or at least six of the nucleic acids or polypeptides are present in the sample.

37. The method of any one of claims 31-36, wherein all of the assays are performed simultaneously to determine if any of the nucleic acids or polypeptides is present in the sample.

38. The method of any one of claims 31-37, further comprising determining whether the subject from whom the sample is obtained is heterozygous or homozygous for any of the nucleic acids.

39. The method of any one of claims 31-38, wherein the assays are performed by microarray, microchip, assay plate, or nanochip analysis.

40. The method of any one of claims 31-39, wherein the assays are performed on a microarray, microchip, assay plate, or nanochip comprising one or more nucleic acid probes, wherein each probe can separately hybridize to one of the nucleic acids under stringent or highly stringent hybridization conditions.

41. The method of any one of claims 31-40, wherein the assays are performed on a microarray, microchip, assay plate, or nanochip comprising one or more antibodies, wherein each antibody separately and selectively can bind to one of the polypeptides.
42. The method of any one of claims 31-41, wherein performing the assays comprises subjecting the test sample, or components extracted from the test sample, to one or more of the assays comprising single nucleotide polymorphism (SNP) analysis, polymerase chain reaction (PCR), quantitative nucleic acid amplification reactions (e.g., quantitative polymerase chain reaction), primer extension analysis, nucleic acid hybridization, nucleic acid sequencing, Northern blot analysis, immunoassay, immunosorbant assay (ELISA), radioimmunoassay (RIA), immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, immunoblotting, mass spectrometry, gel electrophoresis, or a combination thereof.

43. The method of any one of claims 31-42, wherein obtaining the test comprises directly withdrawing the test sample from the subject, or indirectly receiving or evaluating (e.g., assaying) the test sample from a person or entity who has withdrawn the test sample from the subject.

44. A device comprising a solid substrate and binding entities or probes for detecting 2-14 different polypeptide or nucleic acid segments, respectively, wherein each of the binding entities and/or probes selectively binds to one of the following targets:
   a) ADRB1 as a target:
      i. wherein the ADRB1 polypeptide has a serine or glycine at position 49;
      ii. wherein the ADRB1 polypeptide has an arginine or glycine at position 389;
      iii. wherein the ADRB1 nucleic acid comprises rs1801252 (SEQ ID NO:2) with an adenine or guanine at the rs1801252 variable position; and/or
      iv. wherein the ADRB1 nucleic acid comprises rs1801253 (SEQ ID NO:3) with a cytosine or guanine at the rs1801253 variable position;
   b) ADRB2 as a target:
i. wherein the ADRB2 (β₂AR) polypeptide has a glycine or arginine at position 16;
ii. wherein the ADRB2 (β₂AR) polypeptide has glutamic acid or glutamine at position 27;
iii. wherein the ADRB2 nucleic acid comprises rs1042713 (SEQ ID NO:6) with or an adenine or a guanine at the rs1042713 variable position; and/or
iv. wherein the ADRB2 nucleic acid comprises rs1042714 (SEQ ID NO:7) with a cytosine or a guanine at the rs1042714 variable position;

c) CYP2D6 as a target:
   i. wherein the CYP2D6 nucleic acid encodes a CYP2D6-defective gene product; and/or
   ii. wherein the CYP2D6 nucleic acid comprises rs3892097 (SEQ ID NO:10) with an adenine or a guanine at the rs3892097 variable position;

d) angiotensin (AGT) as a target:
   i. wherein the angiotensin polypeptide has methionine or threonine at position 268; and/or
   ii. wherein the angiotensin (AGT) nucleic acid comprises rs699 (SEQ ID NO:14) with cytosine or thymine at the AGT variable position;

e) angiotensin receptor type-I (AGTIR) as a target, wherein the angiotensin receptor type-I (AGTIR) nucleic acid comprises rs5186 (SEQ ID NO:16) at least partially in an AGTIR intron, with an adenine or a cytosine at the rs5186 variable position;

f) renin as a target, wherein the renin nucleic acid comprises rs12750834 (SEQ ID NO:19) with an adenine or a guanine at the rs12750834 variable position;

g) Na⁺ channel (SCNN1A) as a target:
   i. wherein the Na⁺ channel (SCNN1A) polypeptide has threonine or alanine at about position 663 or at about position 722; and/or
ii. wherein the Na\(^{+}\) channel (SCNN1A) nucleic acid comprises rs2228576 (SEQ ID NO:22) with an adenine or a guanine at the rs2228576 variable position;

h) alpha adducin as a target;

i. wherein the alpha adducin polypeptide has glycine or tryptophan at position 460; and/or

ii. wherein the alpha adducin (ADD1) nucleic acid comprises rs4961 (SEQ ID NO:27) with a guanine or thymine at the rs4961 variable position;

i) sodium (Na\(^{+}\)) chloride (Cl\(^{-}\)) co-transporter (SLC12A3) as a target:

i. wherein the sodium (Na\(^{+}\)) chloride (Cl\(^{-}\)) co-transporter (SLC12A3) polypeptide with glycine or alanine at about position 264; and/or

wherein the sodium (Na\(^{+}\)) chloride (Cl\(^{-}\)) co-transporter (SLC12A3) nucleic acid comprises rs1529927 (SEQ ID NO:30) with a cytosine or guanine at the rs1529927 variable position 264; and/or

j) WNK1 as a target:

i. wherein the WNK1 nucleic acid comprises rs2107614 (SEQ ID NO:33) with a cytosine or thymine at the rs2107614 variable position;

ii. wherein the WNK1 nucleic acid comprises rs159744 (SEQ ID NO:34) with a cytosine or guanine at the rs1 59744 variable position.


46. The device of claim 44, comprising binding entities for detecting 3-18, 4-18, 5-18, 6-18, 3-18, 7-18, 8-18, 9-18, 10-18, 11-18, 12-18, 13-18, 14-18,

47. The device of any one of claims 44-46, wherein the solid substrate is a microarray, microchip, assay plate, or nanochip.

48. The device of any one of claims 44-47, wherein the probes are covalently bound to the solid substrate.

49. The device of any one of claims 44-48, wherein the probes are arrayed on the solid substrate, and each probe has a specified, known location.

50. The device of any one of claims 44-47, wherein the antibodies are covalently bound to the solid substrate.

51. The device of any one of claims 44-47 and 50, wherein the binding entities are arrayed on the solid substrate, and each binding entity has a specified, known location.

52. A method comprising:
   (a) obtaining a test sample from a subject;
   (b) determining whether the test sample comprises:
       1. a WNK1 nucleic acid with a cytosine at the variable position of rs1 159744;
       2. a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;
       3. an ADD1 nucleic acid with a thymine at the variable position of rs4961;
       4. a SLC12A3 nucleic acid with a thymine at the variable position of rs529927;
       5. a CYP2D6 nucleic acid with an adenine at the variable position of Rs3892097;
       6. an ADRB1 nucleic acid with a cytosine at the variable position of rs1801253;
7. an ADRB1 nucleic acid with an adenine at the variable position of rs1801252;
8. a renin nucleic acid with a cytosine at the variable position of rs2750834;
9. an AGT1R nucleic acid with a cytosine at the variable position of rs5186;
10. an ACE nucleic acid with a deletion in rs1799752;
11. an AGT nucleic acid with a cytosine at the variable position of rs699;
12. a SCNN1A nucleic acid with an adenine at the variable position of rs2228576;
13. an ADRB2 nucleic acid with a guanine at the variable position of rs1042714; and
14. an ADRB2 nucleic acid with a guanine at the variable position of rs1042713; and

(c) administering a loop diuretic to the subject as a first line therapy, without a beta blocker and without a vasodilator, if the test sample comprises a WNK1 nucleic acid with a cytosine at the variable position of rs1159744 or rs2107614;

(d) administering a hydrochlorothiazide to the subject as a first line therapy, without a beta blocker and without a vasodilator, if the test sample comprises an ADD1 nucleic acid with a thymine at the variable position of rs4961, or if the test sample comprises a SLC12A3 nucleic acid with a thymine at the variable position of rs529927;

(e) administering a hydrochlorothiazide to the subject as a first line therapy, without a beta blocker and without a vasodilator, if the test sample comprises an ADD1 nucleic acid with a thymine at the variable position of rs4961, or if the test sample comprises a SLC12A3 nucleic acid with a thymine at the variable position of rs529927;

(f) if the test sample does not comprise:
1. a WNK1 nucleic acid with a cytosine at the variable position of rs1159744;
2. a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;
3. an ADD1 nucleic acid with a thymine at the variable position of rs4961; or
4. a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927;

I. then administer a beta-blocker drug to the subject as a first line therapy, without a diuretic and without a hydrochlorothiazide, if the test sample comprises:

1. a CYP2D6 nucleic acid with an adenine at the variable position of Rs3892097;

2. an ADRB1 nucleic acid with a cytosine at the variable position of rs1801253;

3. an ADRB1 nucleic acid with an adenine at the variable position of rs1801252;

4. an ADRB2 nucleic acid with a guanine at the variable position of rs1042714; or

5. 2. an ADRB2 nucleic acid with a guanine at the variable position of rs1042713; or

II. then administer an angiotensin II receptor blocker as a first line therapy, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the test sample comprises:

1. a renin nucleic acid with a cytosine at the variable position of rs12750834; or

2. an AGT1R nucleic acid with a cytosine at the variable position of rs5186; or

III. then administer an ACE inhibitor without an angiotensin II receptor blocker as a first line therapy, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the test sample comprises:

1. an ACE nucleic acid with a deletion in rs1799752; or

2. an AGT nucleic acid with a cytosine at the variable position of rs699; or

IV. then administer an amiloride as a first line therapy without an ACE inhibitor, without an angiotensin II receptor blocker, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the test
sample comprises a SCN1A nucleic acid with an adenine at the variable position of rs2228576.

53. The method of claim 52, further comprising administering a second line therapy drug after administration of the first line therapy for at least 1 month, wherein the second line therapy drug is selected from the group consisting of diuretic, a beta-blocker, an ACE inhibitor, a vasodilator, and a combination thereof.

54. A kit comprising: binding entities or probes for detecting 2-14 different polypeptide or nucleic acid segments, respectively, wherein each of the binding entities and/or probes selectively binds to one of the following targets:
   a) ADRBl as a target:
      1. wherein the ADRBl polypeptide has a serine or glycine at position 49;
      2. wherein the ADRBl polypeptide has an arginine or glycine at position 389;
      3. wherein the ADRBl nucleic acid comprises rs1801252 (SEQ ID NO:2) with an adenine or guanine at the rs1801252 variable position; and/or
      4. wherein the ADRBl nucleic acid comprises rs1801253 (SEQ ID NO:3) with a cytosine or guanine at the rs1801253 variable position;
   b) ADRB2 as a target:
      1. wherein the ADRB2 (β2AR) polypeptide has a glycine or arginine at position 16;
      2. wherein the ADRB2 (β2AR) polypeptide has glutamic acid or glutamine at position 27;
      3. wherein the ADRB2 nucleic acid comprises rs1042713 (SEQ ID NO:6) with or an adenine or a guanine at the rs1042713 variable position; and/or
      4. wherein the ADRB2 nucleic acid comprises rs1042714 (SEQ ID NO:7) with a cytosine or a guanine at the rs1042714 variable position;
c) CYP2D6 as a target:
   1. wherein the CYP2D6 nucleic acid encodes a CYP2D6-defective gene product; and/or
   2. wherein the CYP2D6 nucleic acid comprises rs3892097 (SEQ ID NO: 10) with an adenine or a guanine at the rs3892097 variable position;

d) angiotensin (AGT) as a target:
   1. wherein the angiotensin polypeptide has methionine or threonine at position 268; and/or
   2. wherein the angiotensin (AGT) nucleic acid comprises rs699 (SEQ ID NO: 14) with cytosine or thymine at the AGT variable position;

e) angiotensin receptor type-I (AGTIR) as a target, wherein the angiotensin receptor type-I (AGTIR) nucleic acid comprises rs5186 (SEQ ID NO: 16) at least partially in an AGTIR intron, with an adenine or a cytosine at the rs5186 variable position;

f) renin as a target, wherein the renin nucleic acid comprises rs12750834 (SEQ ID NO: 19) with an adenine or a guanine at the rs12750834 variable position;

 g) Na\(^+\) channel (SCNN1A) as a target:
   1. wherein the Na\(^+\) channel (SCNN1A) polypeptide has threonine or alanine at about position 663 or at about position 722; and/or
   2. wherein the Na\(^+\) channel (SCNN1A) nucleic acid comprises rs2228576 (SEQ ID NO:22) with an adenine or a guanine at the rs2228576 variable position;

h) alpha adducin as a target:
   1. wherein the alpha adducin polypeptide has glycine or tryptophan at position 460; and/or
   2. wherein the alpha adducin (ADD1) nucleic acid comprises rs4961 (SEQ ID NO:27) with a guanine or thymine at the rs4961 variable position;

i) sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (SLC12A3) as a target:
1. wherein the sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (SLC12A3) polypeptide with glycine or alanine at about position 264; and/or

2. wherein the sodium (Na\(^+\)) chloride (Cl\(^-\)) co-transporter (SLC12A3) nucleic acid comprises rs1529927 (SEQ ID NO:30) with a cytosine or guanine at the rs1529927 variable position 264; and/or

k) WNK1 as a target:

1. wherein the WNK1 nucleic acid comprises rs2107614 (SEQ ID NO:33) with a cytosine or thymine at the rs2107614 variable position;

2. wherein the WNK1 nucleic acid comprises rs159744 (SEQ ID NO:34) with a cytosine or guanine at the rs159744 variable position; and

instructions for performing an assay to determine whether the test sample comprises any of the polypeptide or the nucleic acid target segments.

55. The kit of claim 54, comprising binding entities or probes for detecting 3-46, 4-46, 5-46, 6-46, 7-46, 8-46, 9-46, 10-46 different polypeptides or nucleic acid segments.

56. The kit of claim 54 or 55, wherein one or more of the binding entities or probes are covalently linked to a distinct label.

57. The kit of any one of claims 54-56, wherein the binding entities or probes are covalently bound to a microarray, microchip, assay plate, or nanochip.

58. The kit of any one of claims 54-57, wherein the instructions describe procedures for determining whether a subject from whom the sample is obtained is heterozygous or homozygous for any of the nucleic acid targets.

59. The kit of any one of claims 54-58, wherein the instructions describe procedures for performing assays on a test sample, or components extracted.
from the test sample, and wherein one or more of the assays comprise single nucleotide polymorphism (SNP) analysis, polymerase chain reaction (PCR), quantitative nucleic acid amplification reactions (e.g., quantitative polymerase chain reaction), primer extension analysis, nucleic acid hybridization, nucleic acid sequencing, Northern blot analysis, immunoassay, immunosorbant assay (ELISA), radioimmunoassay (RIA), immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, immunoblotting, mass spectrometry, gel electrophoresis, or a combination thereof.

60. A method comprising:

(d) administering a loop diuretic to a subject as a first line therapy, without a beta blocker and without a vasodilator, if the subject's genome comprises a WNK1 nucleic acid with a cytosine at the variable position of rs1 159744 or rs2107614;

(e) administering a hydrochlorothiazide to a subject as a first line therapy, without a beta blocker and without a vasodilator, if the subject's genome comprises an ADD1 nucleic acid with a thymine at the variable position of rs4961, or if the test sample comprises a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927; or

(f) administering a hydrochlorothiazide to a subject as a first line therapy, without a beta blocker and without a vasodilator, if the subject's genome comprises an ADD1 nucleic acid with a thymine at the variable position of rs4961, or if the test sample comprises a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927.

61. A method comprising: administering a beta-blocker drug to a subject as a first line therapy, without a diuretic and without a hydrochlorothiazide, if the subject's genome does not comprise:

(e) a WNK1 nucleic acid with a cytosine at the variable position of rs1 159744;

(f) a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;

(g) an ADD1 nucleic acid with a thymine at the variable position of rs4961; or
(h) a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927

but the subject’s genome does comprise:

1. a CYP2D6 nucleic acid with an adenine at the variable position of Rs3892097;

2. an ADRB1 nucleic acid with a cytosine at the variable position of rs1801253;

3. an ADRB1 nucleic acid with an adenine at the variable position of rs1801253;

4. an ADRB2 nucleic acid with a guanine at the variable position of rs1042714; or

5. an ADRB2 nucleic acid with a guanine at the variable position of rs1042713.

62. A method comprising: administering an angiotensin II receptor blocker to a subject as a first line therapy, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the subject’s genome does not comprise:

(a) a WNK1 nucleic acid with a cytosine at the variable position of rs159744;

(b) a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;

(c) an ADD1 nucleic acid with a thymine at the variable position of rs4961; or

(d) a SLC12A3 nucleic acid with a thymine at the variable position of rs1529927

but the subject’s genome does comprise:

1. a renin nucleic acid with a cytosine at the variable position of rs2750834; or

2. an AGTIR nucleic acid with a cytosine at the variable position of rs5186.

63. A method comprising: administering an ACE inhibitor to a subject without an angiotensin II receptor blocker as a first line therapy, without
a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the subject's genome does not comprise:

(a) WNK1 nucleic acid with a cytosine at the variable position of rs159744;

(b) a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;

(c) an ADD1 nucleic acid with a thymine at the variable position of rs4961; or

(d) a SLC12A3 nucleic acid with a thymine at the variable position of rs529927

but the subject's genome does comprise:

1. an ACE nucleic acid with a deletion in rs1799752; or
2. an AGT nucleic acid with a cytosine at the variable position of rs699.

64. A method comprising: administering an amiloride as a first line therapy to a subject without an ACE inhibitor, without an angiotensin II receptor blocker, without a diuretic, without a hydrochlorothiazide, and without a beta-blocker, if the subject's genome does not comprise:

5. a WNK1 nucleic acid with a cytosine at the variable position of rs159744;

6. a WNK1 nucleic acid with a cytosine at the variable position of rs2107614;

7. an ADD1 nucleic acid with a thymine at the variable position of rs4961; or

8. a SLC12A3 nucleic acid with a thymine at the variable position of rs529927.

but if the subject's genome does comprise a SCNN1A nucleic acid with an adenine at the variable position of rs2228576.

65. The method of any of claims 60-64, further comprising administering a second line therapy drug after administration of the first line therapy for at least 1 month, wherein the second line therapy drug is selected from the group
consisting of diuretic, a beta-blocker, an ACE inhibitor, a vasodilator, and a combination thereof.
FIG. 4

DNA YIELD AND PURITY ASSAYS

PASS YIELD AND PURITY ASSAYS

DNA STOCKS STORAGE

FUTURE TESTING

DILUTION PLATE

PCR QA ASSAY

FIG. 5

PASS PCR QA ASSAY

SEQUENOM GENOTYPING

SANGER SEQUENCING

GEL SIZING

REPORTING
**A. CLASSIFICATION OF SUBJECT MATTER**

INV. G01N33/68 C12Q1/68

ADD.

According to International Patent Classification (IPC) or both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, COMPENDEX, EMBASE, FSTA, INSPEC, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>wo 02/071070 A2 (UNIV FLORIDA [US]; JOHNSON JULI E A [US]) 12 September 2002 (2002-09-12) example 4</td>
<td>1-21, 30-51, 54-59, 61.65</td>
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<td>A</td>
<td>wo 2011/048033 A2 (ROSTAQ0 S P A [IT]; BIANCHI GIUSEPPE [IT]; FERRARI PATRIZIA [IT]; MAC) 28 April I 2011 (2011-04-28) abstract; cl aim 6</td>
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which may be considered to establish the publication date of another citation or other special reason (as specified).

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same patent family

Date of the actual completion of the international search

23 July 2015

Date of mailing of the international search report

21/10/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Jacques, Patri ce
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INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As a result all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   52, 53 (completely) ; 1-51, 54-59, 61, 65 (partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 52, 53 (completely) 1-51, 54-59, 61, 65 (partially) Related to genetic variants present in gene ADRB1,

2. claims: 1-51, 54-59, 61, 65 (partially) Related to genetic variants present in gene ADRB2,

3. claims: 1-20, 31-51, 54-59, 61, 65 (partially) Related to genetic variants present in gene CYP2D6

4. claims: 1-20, 31-51, 54-59, 63, 65 (partially) Related to genetic variants present in gene AGT,

5. claims: 1-20, 31-51, 54-59, 62, 65 (partially) Related to genetic variants present in gene AGT1R,

6. claims: 1-20, 31-51, 54-59, 62, 65 (partially) Related to genetic variants present in gene renin,

7. claims: 1-20, 31-51, 54-59, 64, 65 (partially) Related to genetic variants present in gene SCNNIA,

8. claims: 1-20, 31-51, 54-65 (partially) Related to genetic variants present in gene ADD1,

9. claims: 1-20, 31-51, 54-65 (partially) Related to genetic variants present in gene SLC12A3,

10. claims: 1-20, 31-51, 54-65 (partially) Related to genetic variants present in gene WNK1,

11. claims: 63, 65 (partially)
Relating to genetic variants present in gene ACE.
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