A Genetic Variant in the Distal Enhancer Region of the Human Renin Gene Affects Renin Expression

Yasukazu Makino¹, Tadashi Konoshita¹*, Atsuhito Omori¹, Nobuhiro Maegawa¹, Takahiro Nakaya¹, Mai Ichikawa¹, Katsushi Yamamoto¹, Shigeyuki Wakahara¹, Tamotsu Ishizuka¹, Tamehito Onoe², Hiroyuki Nakamura³, Genomic Disease Outcome Consortium (G-DOC) Study Investigators¶

¹ Third Department of Internal Medicine, University of Fukui, Faculty of Medical Sciences, Fukui, Japan, 2 Division of Rheumatology, Department of Internal Medicine, Kanazawa University, Graduate School of Medical Science, Kanazawa, Japan, 3 Department of Environmental and Preventive Medicine, Kanazawa University, Graduate School of Medical Science, Kanazawa, Japan

¶ The complete membership of the Genomic Disease Outcome Consortium (G-DOC) Study Investigators can be found in the Acknowledgments.

* konosita@u-fukui.ac.jp

Abstract

Background

The high heritability of plasma renin activity was confirmed in recent investigations. A variation located near the strong enhancer of the human renin gene (REN), C-5312T, has been shown to have different transcription activity levels depending on its allele: the 5312T allele shows transcription levels that are 45% greater than those of the 5312C allele. The purpose of this study was to confirm the hypothesis that variations in the enhancer region of the REN gene are involved in regulating renal expression of renin.

Methods

Sixty-four subjects with biopsy-proven renal diseases were included in this study (male/female: 35/29, age 41.9 ± 20.9 years, SBP/DBP 123.1 ± 23.7/73.4 ± 14.8 mmHg, s-Cr 0.93 ± 0.63 mg/dl). A genetic variant of REN, C-5312T, was assayed by PCR-RFLP and the TaqMan method. Total RNAs from a small part of the renal cortex were reverse-transcribed and amplified for REN and GAPDH with a real-time PCR system.

Results

Logarithmically transformed expression values of the relative ratio of REN to GAPDH (10⁻³) were as follows (mean ± SE): CC (26 cases), 0.016 ± 0.005; CT (33 cases), 0.047 ± 0.021 (p = 0.41 vs. CC); TT (5 cases), 0.198 ± 0.194 (p = 0.011 vs. CC, p < 0.031 vs. CT). Thus, significant differences in REN expression were observed among the genetic variants.
Conclusion
The results suggest that variants in the enhancer region of the human renin gene have an effect on the expression levels of renin in renal tissue; this observation is in good accordance with the results of the transcriptional assay.

Introduction
The renin-angiotensin system (RAS) plays pivotal roles in blood pressure regulation,[1] cardiovascular, renal and metabolic conditions[2–4] and pharmacological status.[5, 6] One of the major rate-limiting steps of this system is the conversion of angiotensinogen to angiotensin I, which is catalyzed by renin. Thus, the regulation of renin gene expression is thought to be fundamental to the regulation of the total system. Recent studies also confirmed that a higher plasma renin level was associated with greater cardiovascular mortality in patients referred to coronary angiography[7] and in community-based cohort studies.[8] High heritability estimates were reported for plasma renin activity in an investigation.[9] These observations are presumed to be attributed to genetic variants, especially those in the promoter locus that contributes to transcriptional activity. Previously, six protein-binding sites had been mapped and characterized in the proximal promoter region of the human renin gene (REN) (-336 to +16) by DNase I footprint assay with nuclear extracts from human chorionic cells or ischemic human renal cortex.[10–14] These evaluations revealed the physiological mechanisms of the cis-elements; however, there have been no studies on genetic variants in the proximal promoter region. On the other hand, a strong enhancer in the human REN promoter region, located 5777–5552 nucleotides upstream from the transcription start site, has been described.[15] A variant located near this enhancer, C-5312T, has been shown to have a different transcription activity level according to its alleles in choriodcidual cells, a model of renin-producing cells. The levels of transcription were 45% greater with the 5312T variant than with the 5312C variant.[16] Recently, we have demonstrated that variations in the renin gene were associated with plasma renin activity as a genetic factor[17] and also that such variations are pharmacogenetic determinants of the antihypertensive effect of angiotensin receptor blockers.[18, 19]

The purpose of this study was to confirm the hypothesis that this validated enhancer region variant is associated with renal REN expression. Thus, genetic variants and the renal REN mRNA from renal biopsy specimens were assayed.

Methods
Subjects and treatments
This study was undertaken in accordance with the Declaration of Helsinki Principles. The study protocol was approved by the ethics committee of the University of Fukui (No. 17–12, 13–1 and 14–2). Written informed consent to participate in this study was provided by all participants. Subjects were 64 patients with biopsy-proven renal conditions, including 6 with benign nephrosclerosis, 7 with minor lesions, 35 with primary glomerulonephritis, 10 with diabetic lesions, 5 lupus erythematoses and 1 with interstitial nephritis.

For each subject, salt intake was standardized to 6 to 10 g daily during hospitalization according to the hypertension status. The numbers of patients administered with anti-hypertensive agents were as follows: calcium channel blocker (CCB), 8; alpha-blocker (alpha B), 7; diuretics, 5; ACE inhibitor (ACEI), 1; angiotensin receptor blocker (ARB), 1. However, ACEI and ARB were replaced by CCB or alpha B at least 1 week prior to biopsy.
Genetic variant analysis

Genomic DNA was isolated by phenol-chloroform extraction or with a QIAamp DNA Blood Mini Kit (QIAGEN Inc., Tokyo, Japan) from whole blood drawn from subjects with EDTA-2Na. In DNA amplification procedures for the genetic variant assay, a thermal cycler (ASTEC, PC-700, Fukuoka, Japan) was used.

For early samples, the REN, C-5312T, was assayed by PCR-RFLP with DdeI digestion. The primers used were as follow: sense oligonucleotide, 5′-CGTAGTGCCATTHTTTAGGAAC-3′, and anti-sense oligonucleotide, 5′-AACACAAAGCAGGCTTAA-3′. The PCR program consisted of 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds followed by a final extension at 72°C for 5 minutes. Samples were then incubated with DdeI overnight at 37°C. To examine the sizes of the PCR products, after the addition of loading buffer containing 10% glycerol, 10 μl of the mixture was loaded onto agarose gels.

For recent samples, the variant was assayed using the TaqMan method using the common PCR conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA) with a custom-made primer set. The primers used were as follow: sense oligonucleotide, "ACTAGGAATCCAGGAGAATAGGTCTTT" and anti-sense oligonucleotide, "CCTTA-GAACACAAAGCAGGCTTAA". (Fig 1).

Expression assay by real-time quantitative RT-PCR

Renal RNAs were extracted from a very small sample (approximately 2 mm of an 18G needle specimen) of renal cortex tissue obtained by percutaneous renal biopsy under echography. Almost all of these specimens contained approximately 20 to 30 glomeruli. After collecting the biopsy specimens, total RNAs were immediately extracted by using a commercial extraction kit, RNA-Bee (TEL-TEST, Inc., Friendswood, USA), according to the manufacturer’s protocol. The cDNAs were synthesized by reverse transcription with 500 ng/μl of Oligo-dT (TOYOBO Inc., Osaka, Japan) and reverse transcriptase (M-MLV) (TOYOBO Inc., Osaka, Japan). The synthesized cDNAs were amplified for renin as the target gene and GAPDH as a house-keeping gene. The primer sequences were: renin, 5′-GTGTCTGTGGGGTCACTCCACCTTG-3′ (sense) and 5′-GGATTCCTGAAATACATAGTCCGT-3′ (anti-sense); GAPDH, 5′-CCCATCACCTCTTCCAGGAG-3′ (sense) and 5′-GTTGTCATGGATGACCTTGGC-3′ (anti-sense). The real-time PCR reactions took place in a reaction volume of 20 μl containing 0.5 mM of each primer and 2 μl of cDNA as a template in 2× QuantiTect PCR Master Mix (QIAGEN Inc., Tokyo, Japan). Measurements of the specific mRNAs of renin and GAPDH were performed by using a LightCycler System (Roche Diagnostics Inc., Tokyo, Japan). Each sample was measured in duplicate. Absolute quantification was performed using prepared concentration-known control samples (Fig 2). The mRNA levels were expressed as relative values to GAPDH mRNA and then logarithmically transformed.

Statistical analyses

Statistical analyses were performed with SPSS (ver. 17.0; SPSS Japan, Inc., Tokyo, Japan). Data are expressed as numbers, percentages, means ± SD, or medians (interquartile ranges), as appropriate. The differences between the variables of two or three groups were analyzed by ANOVA or Wilcoxon’s signed rank test, as follows: systolic blood pressure (SBP), diastolic blood pressure (DBP), serum creatinine level (s-Cr), eGFR, plasma renin activity (PRA), and plasma and aldosterone concentration (PAC) were analyzed by ANOVA; urinary sodium excretion, urinary potassium excretion, and urinary chloride excretion were analyzed by Wilcoxon’s signed rank test. Logarithmically transformed values of the relative expression of REN compared to that of GAPDH (10^-3) for each genotype were evaluated by ANOVA (Fig 3).
performed comparisons between two genotypic groups as well as between three genotypic groups to better evaluate dominant and recessive models.

Results
Basic clinical characteristics of the subjects
The basic clinical characteristics of the subjects were as follows (Table 1) (values are means ± SD or means (interquartile), as appropriate): age, 41.9 ± 20.9 years; SBP, 123.1 ± 23.7 mmHg; DBP,
73.4 ± 14.8 mmHg; s-Cr, 0.93 ± 0.63 mg/dl; eGFR, 83.4 ± 35.3 ml/min/1.73 m²; PRA, 2.7 ± 1.3 ng/ml/h; PAC, 91.6 ± 49.7 pg/ml; urinary albumin excretion, 266.7 (44.2 – 682.8) mg/gCr; urinary sodium excretion, 105.3 (83.5 – 157.9) mEq/gCr; and urinary potassium excretion, 30.9 (25.6 – 38.5) mEq/gCr. Other major clinical characteristics are also listed in Table 1.

Genetic variants and clinical characteristics

The prevalence of the C-5312T genotypes were as follows: CC, 26 cases; CT, 33 cases; TT, 5 cases. The distributions were similar to those predicted from the Hardy Weinberg equilibrium. The main characteristics of each genotype are shown in Table 2. No significant difference was observed in these clinical characteristics among genotypes.
Genetic variants and the gene expression in renal tissue of REN

REN expression was measured at $10^{-3}$ order that of GAPDH expression. The values were as follow (means ± SE): CC, 0.016 ± 0.005; CT, 0.047 ± 0.021 (p = 0.41 vs. CC); TT, 0.198 ± 0.194 (p = 0.011 vs. CC, p < 0.031 vs. CT) (A). The results of the comparisons between genotypes were as follows: CC and CT/TT (0.067 ± 0.031), p = 0.177 (B); CC/CT (0.033 ± 0.012) and TT, p = 0.015 (C).

doi:10.1371/journal.pone.0137469.g003

Table 1. Subjects characteristics.*

| Characteristics                  |       |
|----------------------------------|-------|
| Number                           | 64    |
| Female gender—no. (%)            | 29 (45.3%) |
| Age—yr                          | 41.9±20.9 |
| Body-mass index†                 | 23.5±3.9 |
| Blood pressure, mmHg             |       |
| Systolic                         | 123.1±23.7 |
| Diastolic                        | 73.4±14.8 |
| Serum sodium—mEq/l               | 139.9±2.8 |
| Serum potassium—mEq/l            | 4.1±0.3 |
| Serum chloride—mEq/l             | 103.4±3.0 |
| Serum creatinine—mg/dl           | 0.93±0.63 |
| eGFR—ml/min/1.73m²               | 83.4±35.3 |
| Plasma renin activity, ng/ml/hr   | 1.7±1.3 |
| Plasma aldosterone concentration, pg/ml | 91.6±49.7 |
| Urinary albumin excretion—mEq/creatinine‡ | 266.7 (44.2–682.8) |
| Urinary sodium excretion—mEq/creatinine‡ | 105.3 (83.5–157.9) |
| Urinary potassium excretion—mEq/creatinine‡ | 30.9 (25.6–38.5) |
| Urinary chloride excretion—mEq/creatinine‡ | 105.8 (77.7–148.1) |

*Plus-minus values are means ± SD.
†The body-mass index is the weight in kilograms divided by square of the height in meters.
‡Values shown are medians (interquartile ranges).
eGFR: estimated glomerular filtration rate.

doi:10.1371/journal.pone.0137469.t001
(p = 0.011 vs. CC, p < 0.031 vs. CT) (Fig 3). For the comparison between C allele homozygotes and CT/TT, the values were 0.016 ± 0.005 and 0.067 ± 0.031, respectively (p = 0.177). For the comparison between CC/CT and T allele homozygotes, the values were 0.033 ± 0.012 and 0.198 ± 0.194, respectively (p = 0.015). After Bonferroni’s correction for the examination of three genotypic groups (CC, CT, and TT), the difference between CC and TT was significant. Thus, a significant difference in REN expression in renal tissue was observed between genetic variants of C-5312T.

Discussion

Recently, genetic variants have been studied for association with their phenotypic characteristics in a large number of physiological and pathological conditions. In particular, variants of the components of RAS have been evaluated in cardiovascular and renal disorders.[20–26] These early emerging variants have been validated mainly by evaluating plasma concentrations as intermediate phenotypes.[21, 27] However, only a small number of recent reports have evaluated the validity of variants. The elucidation of the tissue expression levels of targeted genes is thought to be informative for such validation of genetic variants. Renin gene expression in human renal tissues has been evaluated quantitatively,[28] but no such study has been conducted with genetic variants. REN C-5312T is one of the rare validated genetic variants with reports that have shown their effects at the genetic transcriptional level[16] and also at the plasma activity level.[17] In this study, we evaluated the renal expression level of REN in human renal tissues in respect to REN genetic variants for the first time. Tissue expression levels of REN were significantly higher in T allele homozygotes than in heterozygotes and C allele homozygotes. This observation is in good accordance with the results of the transcriptional assay. Thus, C-5312T can now be regarded as validated in tissue expression level as well as in transcriptional level and plasma activity level.

Renin is thought to be excreted via a regulated pathway and its excretion and synthesis is largely affected by physiological status, especially the posture.[29] At renal biopsy, our subjects were prone for at least 30 minutes before the renal specimens were taken so that our

### Table 2. Genetic variations in C-5312T and clinical characteristics.*

| Characteristics                        | CC       | CT       | TT       |
|----------------------------------------|----------|----------|----------|
| Number                                 | 26       | 33       | 5        |
| Blood pressure, mmHg                   |          |          |          |
| Systolic                               | 123.0±23.1 | 123.8±24.7 | 119.0±23.8 |
| Diastolic                              | 74.5±13.9 | 72.9±16.0 | 71.2±14.3 |
| Serum creatinine—mg/dl                 | 0.85±0.41 | 1.01±0.79 | 0.75±0.10 |
| eGFR—ml/min/1.73m²                     | 87.4±37.2 | 80.0±34.9 | 85.8±31.2 |
| Plasma renin activity, ng/ml/hr        | 1.8±1.6  | 1.6±1.2  | 1.9±0.7  |
| Plasma aldosterone concentration, pg/ml| 90.4±48.7 | 94.1±54.2 | 81.2±19.9 |
| Urinary sodium excretion—mEq/creatinine†| 106.3(99.1–159.2) | 101.9(80.3–154.4) | 107.3(94.4–128.8) |
| Urinary potassium excretion—mEq/creatinine†| 32.9(27.3–44.1) | 29.6(24.0–36.5) | 35.6(32.1–60.8) |
| Urinary chloride excretion—mEq/creatinine†| 114.1(89.5–162.9) | 105.3(74.7–145.3) | 104.7(101.2–149.3) |

*Plus-minus values are means ± SD.
†Values shown are medians (interquartile ranges).
eGFR: estimated glomerular filtration rate.

doi:10.1371/journal.pone.0137469.t002
measurements of mRNA would represent the expression levels of the basal status. Consequently, C-5312T is thought to be related to the expression of REN at the basal status. It is possible that physiologically exaggerated status, for example, standing, alters the relationship between the genotype and gene expression.

High heritability estimates were reported for plasma renin activity in a recent investigation,[9] and we have also demonstrated in a recent report that a renin gene variant was associated with plasma renin activity as a genetic factor [17]. Each PRA value by genotype for C-5312T tended to be in fair accordance with the tissue expression levels; however, they did not reach the level of statistical significance in this study. Associations between genetic variants of REN and hypertension were not recognized in early studies,[30–32], but in recent studies, they have been recognized for several conditions including hypertension,[33, 34] primary aldosteronism [35] and diabetic nephropathy.[36]

Several limitations of the present study should be described. Although the tissue renin expression levels were log-transformed, the distribution was still not normal. The sample number in this study was relatively small, so differences in disease states could not be assessed by genotype. Cessation of ACEI and ARB for 1 week may have been sufficient to normalize renin expression. Although a sample population comprising individuals with various genetic backgrounds would have been ideal, this study comprised only Japanese subjects. One other limitation was that we investigated only one variant of REN.

In conclusion, the results suggest that variants in the enhancer region of the human renin gene have an effect on the expression levels of renin in renal tissue; this observation is in good accordance with the results of the transcriptional assay. Thus, the validated C-5312T of REN may be a candidate gene for susceptibility to cardiovascular, renal and metabolic conditions. Closer examination in a larger population is necessary to solve these issues.

Supporting Information

S1 File. All raw data of the subjects.

(XLSX)

Acknowledgments

We are grateful to the study investigators of the Genomic Disease Outcome Consortium (G-DOC) Investigators, which was organized as the Kanazawa Renal Disease Study Group in 1995 for presenting a part of the subjects of this study. The original members of this group were: Katsuoh Haruki (Haruki Clinic, Komatsu, Japan), Shuichi Hatakeyama (Hatakeyama Clinic, Oyabe, Japan), Masayoshi Hirata (Takaoka Municipal Hospital, Takaoka, Japan), Masao Imura (Imura Internal Medical Office, Hakusan, Japan), Masanori Ito (Fukui Red Cross Hospital, Fukui, Japan), Masahiro Kuroda (Asanagi Hospital, Takaoka, Japan), Hiroshi Matsumoto (Wajima Municipal Hospital, Wajima, Japan), Hiroaki Muramoto (Japan Community Health Care Organization Kanazawa Hospital, Kanazawa, Japan), Tamehito Onoe (Division of Rheumatology, Department of Internal Medicine, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan), Ichiro Koni (Koni Clinic, Uchinada, Japan), Ryoichi Miyazaki (Fujita Memorial Hospital, Fukui, Japan), Yohei Tofuku (Wajima Municipal Hospital, Wajima, Japan), and Ryoyu Takeda (Professor Emeritus, Kanazawa University, Kanazawa, Japan). The lead author of the group is Tadashi Konoshita (konosita@u-fukui.ac.jp). We are also grateful to Ms. Yoshimi Kubo, Ms. Yoko Hayashida and Ms. Tomoko Iwamoto for their secretarial and technical assistance.
Author Contributions

Conceived and designed the experiments: YM TK. Performed the experiments: YM TK AO NM. Analyzed the data: YM TK HN. Contributed reagents/materials/analysis tools: YM TK TN MI KY SW TI TO. Wrote the paper: YM TK.

References

1. Corvol P, Soubrier F, Jeunemaitre X. Molecular genetics of the renin-angiotensin-aldosterone system in human hypertension. Pathol Biol (Paris). 1997; 45(3):229–39. PMID: 16567826

2. Konoshita T, Wakahara S, Mizuno S, Motomura M, Aoyama C, Makino Y, et al. Tissue gene expression of renin-angiotensin system in human type 2 diabetic nephropathy. Diabetes Care. 2006; 29(4):848–52. PMID: 16567826

3. Wakahara S, Konoshita T, Mizuno S, Motomura M, Aoyama C, Makino Y, et al. Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio. Endocrinology. 2007; 148(5):2453–7. Epub 2007/02/17. doi:10.1210/en.2006-1287 PMID: 17303661

4. Ichikawa M, Konoshita T, Nakaya T, Yamamoto K, Yamada M, Sato S, et al. Genetic variant of the renin-angiotensin system and prevalence of type 2 diabetes mellitus: a modest but significant effect of aldosterone synthase. Acta Diabetol. 2014; 51(4):595–9. Epub 2014/02/20. doi:10.1007/s00592-014-0561-7 PMID:24549414

5. Konoshita T, Makino Y, Kimura T, Fujii M, Wakahara S, Arakawa K, et al. A new-generation N/L-type calcium channel blocker leads to less activation of the renin-angiotensin system compared with conventional L type calcium channel blocker. J Hypertens. 2010; 28(10):2156–60. Epub 2010/07/14. doi:10.1097/HJH.0b013e32833d01dd PMID:20625317

6. Konoshita T, Makino Y, Kimura T, Fujii M, Morikawa N, Wakahara S, et al. A crossover comparison of urinary albumin excretion as a new surrogate marker for cardiovascular disease among 4 types of calcium channel blockers. Int J Cardiol. 2013. Epub 2011/11/25. S0167-5273(11)02024-9 [pii] doi:10.1016/j.ijcard.2011.10.133 PMID:22112682

7. Tomaschitz A, Pilz S, Ritz E, Morganti A, Grammer T, Amrein K, et al. Associations of plasma renin with 10-year cardiovascular mortality, sudden cardiac death, and death due to heart failure. Eur Heart J. 2011; 32(21):2642–9. Epub 2011/05/25. ehr150 [pii] doi:10.1093/eurheartj/ehr150 PMID: 21606079

8. Daimon M, Konta T, Oizumi T, Karasawa S, Kaino W, Takase K, et al. Higher plasma renin activity is a risk factor for total mortality in older Japanese individuals: the Takahata study. Metabolism. 2012; 61 (4):504–11. Epub 2011/10/18. S0026-0495(11)00270-8 [pii] doi:10.1016/j.metabol.2011.08.004 PMID:22001336

9. Kotchen TA, Kotchen JM, Grim CE, George V, Kaldunski ML, Cowley AW, et al. Genetic determinants of hypertension: identification of candidate phenotypes. Hypertension. 2000; 36(1):7–13. PMID:10904005

10. Borensztein P, Germain S, Fuchs S, Philippe J, Corvol P, Pinet F. cis-regulatory elements and trans-acting factors directing basal and cAMP-stimulated human renin gene expression in chorionic cells. Circ Res. 1994; 74(5):764–73. PMID:8156625

11. Germain S, Konoshita T, Philippe J, Corvol P, Pinet F. Transcriptional induction of the human renin gene by cyclic AMP requires cyclic AMP response element-binding protein (CREB) and a factor binding a pituitary-specific trans-acting factor (Pit-1) motif. Biochem J. 1996; 316(Pt 1):107–13.

12. Konoshita T, Germain S, Philippe J, Corvol P, Pinet F. Evidence that renal and chorionic tissues contain similar nuclear binding proteins that recognize the human renin promoter. Kidney Int. 1996; 50 (5):1515–24. PMID: 8914017

13. Konoshita T, Makino Y, Wakahara S, Ido K, Yoshida M, Kawai Y, et al. Candidate cis-elements for human renin gene expression in the promoter region. J Cell Biochem. 2004; 93(2):327–36. PMID: 15368359

14. Konoshita T, Fuchs S, Makino Y, Wakahara S, Miyamori I. A proximal direct repeat motif characterized as a negative regulatory element in the human renin gene. J Cell Biochem. 2007; 102(4):1043–50. Epub 2007/04/25. doi:10.1002/jcb.21341 PMID:17455195.

15. Germain S, Bonnet F, Philippe J, Fuchs S, Corvol P, Pinet F. A novel distal enhancer confers conifing expression on the human renin gene. J Biol Chem. 1998; 273(39):25292–300. PMID:9737995

16. Fuchs S, Philippe J, Germain S, Mathieu F, Jeunemaitre X, Corvol P, et al. Functionality of two new polymorphisms in the human renin gene enhancer region. J Hypertens. 2002; 20(12):2391–8. PMID:12473863
17. Konoshita T, Nakaya T, Sakai A, Yamada M, Ichikawa M, Sato S, et al. Determinants of plasma renin activity: role of a human renin gene variant as a genetic factor. Medicine (Baltimore). 2014; 93(29):e354. Epub 2014/12/30. doi: 10.1097/md.0000000000000354

18. Konoshita T, Kato N, Fuchs S, Mizuno S, Aoyama C, Motomura M, et al. Genetic variant of the Renin-Angiotensin system and diabetes influences blood pressure response to Angiotensin receptor blockers. Diabetes Care. 2009; 32(8):1485–90. Epub 2009/06/11. dc09-0348 [pii] doi: 10.2337/dc09-0348 PMID: 19509012; PubMed Central PMCID: PMC2713645.

19. Konoshita T. Do genetic variants of the Renin-Angiotensin system predict blood pressure response to Renin-Angiotensin system-blocking drugs?: a systematic review of pharmacogenomics in the Renin-Angiotensin system. Curr Hypertens Rep. 2011; 13(5):356–61. Epub 2011/05/13. doi: 10.1007/s11906-011-0212-0 PMID: 21562941; PubMed Central PMCID: PMC3179582.

20. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, et al. Deletion polymorphism in the angiotensinogen gene: a potential risk factor for myocardial infarction [see comments]. Nature. 1992; 359(6396):641–4. PMID: 1326889

21. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al. Molecular basis of human hypertension: role of angiotensinogen. Cell. 1992; 71(1):169–80. PMID: 1394429

22. Caulfield M, Lavender P, Farrall M, Munroe P, Lawson M, Turner P, et al. Linkage of the angiotensin-converting enzyme gene to essential hypertension [see comments]. N Engl J Med. 1994; 330(23):1629–33. PMID: 8177268

23. Harden PN, Geddes C, Rowe PA, McIlroy JH, Boulton-Jones M, Rodger RS, et al. Polymorphisms in angiotensin-converting-enzyme gene and progression of IgA nephropathy [see comments]. Lancet. 1995; 345(8964):1540–2. PMID: 7791440

24. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease [see comments]. N Engl J Med. 1995; 332(11):706–11. PMID: 7854377

25. Fogarty DG, Harron JC, Hughes AE, Nevin NC, Doherty CC, Maxwell AP. A molecular variant of angiotensin-converting enzyme is a potent risk factor for myocardial infarction [see comments]. Lancet. 1995; 345(8924):1540–2. PMID: 7791440

26. Hackenthal E, Paul M, Ganten D, Taugner R. Morphology, physiology, and molecular biology of renin secretion. Physiol Rev. 1990; 70(4):1067–116. PMID: 2217555

27. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990; 86(4):1343–6. Epub 1990/10/01. doi: 10.1172/JCI14844 PMID: 1976855; PubMed Central PMCID: PMC296868.

28. Waebber B, Brunner HR. Cardiovascular hypertrophy: role of angiotensin II and bradykinin. J Cardiovasc Pharmacol. 1996; 27 Suppl 2:S36–40. PMID: 8723998

29. Hackenthal E, Paul M, Ganten D, Taugner R. Morphology, physiology, and molecular biology of renin secretion. Physiol Rev. 1990; 70(4):1067–116. PMID: 2217555

30. Soubrier F, Jeunemaitre X, Rigat B, Houot AM, Cambien F, Corvol P. Similar frequencies of renin gene restriction fragment length polymorphisms in hypertensive and normotensive subjects. Hypertension. 1990; 16(6):712–7. PMID: 1978831

31. Jeunemaitre X, Rigat B, Charru A, Houot AM, Soubrier F, Corvol P. Sib pair linkage analysis of renin gene haplotypes in human essential hypertension. Hum Genet. 1992; 88(3):301–9. Epub 1994/10/01. doi: 10.1007/bf00095085 PMID: 1257410

32. Mercure C, Thibault G, Lussier-Cacan S, Davignon J, Schiffrin EL, Reudelhuber TL. Molecular analysis of human prorenin prosegment variants in vitro and in vivo. J Biol Chem. 1995; 270(27):16355–9. Epub 1995/07/07. PMID: 7608205

33. Frossard PM, Lestringant GG, Elshahat YI, John A, Obineche EN. An MboI two-allele polymorphism may implicate the human renin gene in primary hypertension. Hypertens Res. 1998; 21(3):221–5. Epub 1998/10/24. PMID: 9786608.

34. Hasimou B, Nakayama T, Mizutani Y, Izumi Y, Asai S, Soma M, et al. Haplotype analysis of the human renin gene and essential hypertension. Hypertension. 2003; 41(2):308–12. Epub 2003/02/08. PMID: 1257410

35. Klemm SA, Ballantine DM, Gordon RD, Tunny TJ, Stowasser M. The renin gene and aldosterone-producing adenomas. Kidney Int. 1994; 46(6):1591–3. PMID: 7700015

36. Deinum J, Tarnow L, van Gool JM, de Bruin RA, Derkx FH, Schalekamp MA, et al. Plasma renin and prorenin and renin gene variation in patients with insulin-dependent diabetes mellitus and nephropathy. Nephrol Dial Transplant. 1999; 14(8):1904–11. Epub 1999/08/26. PMID: 10462269.