The Natural Mutation Y248C of Human Angiotensinogen Leads to Abnormal Glycosylation and Altered Immunological Recognition of the Protein*

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Common molecular variants of the angiotensinogen gene have been associated with human hypertension. The rare Tyr to Cys change at residue 248 of mature angiotensinogen was identified in one pedigree. Heterozygous individuals (Y248C) had a 40% decrease in plasma angiotensinogen concentration and a 35% reduction of the angiotensin I production rate. Recombinant wild-type (Tyr-248) and mutant (Cys-248) proteins were stably expressed in Chinese hamster ovary cells. Angiotensinogen monoclonal antibodies revealed marked differences in the epitope recognition of the mutant protein and allowed the demonstration of its presence in plasma of Y248C individuals. Similar kinetic constants of angiotensin I production with human renin were observed for both proteins. Western blot analysis showed similar heterogeneities; however, a 3-kDa increase in molecular mass for the Cys-248 protein was observed after immunopurification. Metabolic labeling of the intracellular Cys-248 protein showed a 61-kDa band in addition to the 55.5- and 58-kDa bands observed for the Tyr-248 protein, with all bands being sensitive to endoglycosidase H. In addition, pulse-chase studies revealed a slower intracellular processing for the Cys-248 protein. In conclusion, the Cys-248 mutation alters the structure, glycosylation, and secretion of angiotensinogen in Chinese hamster ovary cells and is accompanied by a decrease in plasma angiotensinogen concentration in Y248C individuals.

Angiotensinogen is the natural and specific substrate of the renin-angiotensin system that plays a major role in water and salt homeostasis, vascular tone, and blood pressure regulation. The specific interaction between renin and angiotensinogen is the initial and rate-limiting step of this enzymatic cascade that generates the decapeptide angiotensin I (Ang I),1 which is processed further to the active octapeptide angiotensin II by angiotensin I-converting enzyme. Angiotensinogen is a glycoprotein constitutively secreted by the liver into plasma and extracellular fluid (1, 2). In humans, plasma angiotensinogen concentration is close to the Km for its interaction with renin, and variations of its concentration might therefore influence Ang I generation (3). Pharmacological (4, 5) and genetic (6–8) studies in rodents and in humans (9, 10) have demonstrated a positive relationship between plasma angiotensinogen levels and blood pressure.

The role of angiotensinogen in blood pressure regulation has been highlighted by the linkage observed between a polymorphic marker of the angiotensinogen (AGT) gene and essential hypertension in two large series of hypertensive sibships (11) and two others set of hypertensive families (12, 13). Several molecular variants have been discovered in the AGT gene, and each of these could potentially be responsible for inherited predisposition to human blood pressure variation. Most notably, a common variant (M235T, change from Met to Thr at residue 235) was found to be more frequent in hypertensive probands than in controls (11, 14). Despite strong ethnic differences in Thr-235 allele frequency, these findings have been replicated in other ethnic groups (15, 16), although other studies failed to detect any significant difference in the Thr-235 allele frequency between normotensives and hypertensives (12, 17). Whether Thr-235 directly accounts for a physiological effect or acts as a marker for a causative mutation is as yet unresolved, although its association with a 10–20% increase in plasma angiotensinogen (11, 14, 18) led to the hypothesis that such a common variant could induce a chronic overstimulation of the renin-angiotensin system, which would explain the susceptibility to hypertension.

In addition to this common variant, some rare mutations have been detected in a few hypertensive probands (11). Their clinical and biochemical significance can only be established by analysis of the rare affected individuals and the biochemical characteristics of the mutant protein. As an example, the replacement of leucine 10 by a phenylalanine corresponding to the site of angiotensinogen cleavage by renin and to the C-terminal amino acid of Ang I was found in a woman who had suffered preeclampsia, which significantly alters the kinetics of the enzymatic reactions of both renin and angiotensin I-converting enzyme (19).

In this study, we have investigated the clinical and biochemical significance of the change from a tyrosine to a cysteine at position 248, present in the heterozygous state (Y248C) in several subjects belonging to the same family.
The adrenine to guanine transition at nucleotide +743, resulting in a change from a tyrosine to a cysteine residue at residue 248 of the mature angiotensinogen protein, was identified previously in 1 of 132 hypertensive probands originating from Salt Lake City (11). This initial study was extended in French patients using the same methodology. Genomic DNA was subjected to enzymatic amplification with specific primers of the 3′-part of exon 2 of angiotensinogen, already reported (12). A mutation search was performed with allele-specific oligonucleotides corresponding to the wild-type (Ty-248) (5′-AAACACTCTGC-CACT-3′) and variant (Cys-248) (5′-AGTGGACGCAGGTGTT-3′) alleles. Protocols of enzymatic amplification, denaturation of DNA, fixation on nylon membranes, and hybridization have been described previously (11).

DNA samples from 356 probands of hypertensive Caucasian families selected in the Broussais Hypertension Clinic (20) and 325 normotensive Caucasians were screened. Heterozygosity for the Cys-248 allele was found in one hypertensive individual and in one normotensive individual. Consequently, we screened all members of the hypertensive families for the presence of the Cys-248 mutation (635 subjects in addition to the 356 hypertensive probands). Two positive hypertensive brothers with mild and late onset of hypertension were unavailable for subsequent studies. The other positive subject was an offspring of a hypertensive individual. We extended clinical and biological data from the hypertensive proband originating from Salt Lake City (11). This initial series was extended to the hypertensive probands originating from a large and extended family in Sweden. The amino acid sequence of this human AGT cDNA corresponds to the most frequent haplotype in the Caucasian population (Th-174/Met-235). Site-directed mutagenesis of the tyrosine to position 248 was performed by the method of Kunkel (29) using an in vitro mutagenesis kit (Mutage-Genet Phagmed, Bio-Rad) and the following oligonucleotide: 5′-TCAACACCTGGCTCACTTCC-3′. The entire sequence of the 1.7-kilobase pair of angiotensinogen cDNA was sequenced to confirm the presence of the cysteine mutation and the absence of spurious mutations.

Expression of Ty-248 and Cys-248 Angiotensinogens in CHO Cells

To isolate the coding sequence of Ty-248 and Cys-248 cDNAs, plasmid pTAG1 and pTAG-C248 were digested by XbaI and inserted into the expression vector pECE (30). Each plasmid (10 μg) was cotransfected into CHO cells with a neomycin resistance plasmid (pSV2neo; 2 μg) using the calcium phosphate precipitation procedure (31). CHO cells were grown in Ham's F-12 medium supplemented with 10% fetal calf serum, 10 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. After 48 h, cells were selected with a 750 μM concentration of neomycin analog G418 (Life Technologies, Inc.). Pure cell lines expressing angiotensinogen were obtained by the limiting dilution subcloning technique after selection of the highly expressing clones by direct and enzymatic measurements of the angiotensinogen concentration in the medium. The angiotensinogen concentration by 106 transfected cells in serum-free medium was 1–2 μg/ml/day.

Angiotensinogen Immunopurification Procedure

The CHO cell supernatants were concentrated (Diaflo YM-10 ultrafiltration cell membrane, Amicon, Inc.) to <5 ml and equilibrated in 0.1 M KH2PO4 buffer, pH 7.4, prior to loading an immunopurification column (4.5 cm of purified 181 anti-angiotensinogen mAb immobilized on 1 ml of CNBr-activated Sepharose 4B (Pharmacia)), previously equilibrated in the same buffer. Immunoaffinity resin was washed with at least 10 volumes of 0.1 M KH2PO4 buffer, pH 7.4. Recombinant angiotensinogen was then eluted with 0.1 M sodium acetate, pH 3.5, reconstituted by extensive dialysis against 0.1 M NaH2PO4/H2O buffer, pH 7.4, and concentrated. The angiotensinogen content was measured by angiotensinogen assays and analyzed by Coomassie Blue staining and Western blot analysis.

Enzymatic Kinetics

Samples were incubated with 50 pg of purified human recombinant renin for 1 h at 37 °C in 0.5 ml of 0.2 M Tris-HCl buffer, pH 7.3, containing 150 mM EDTA, 1.4 mM phenylmethylsulfonyl fluoride, and 5% (w/v) human serum albumin. The reaction was stopped by incubation on ice, and Ang I production was quantified by RIA. Two different experiments were performed in duplicate for six different concentrations (0.1–1.0 μM) of angiotensinogen.

Pulse-Chase Experiments of 35S-Labeled Angiotensinogen in CHO Cells

Metabolic labeling of CHO cells was performed as described previously (32) with few modifications. After 1 h of preculture in methionine- and cysteine-free Ham's F-12 medium, cells were labeled for 10 min with 50 μCi of [35S]methionine and [35S]cysteine (pulse) followed by the addition of Ham's F-12 medium at 37 °C (chase). At each time, the medium was collected, and cells were solubilized at 4 °C in buffer containing a mixture of proteinase inhibitors (32) and centrifuged (12,000 × g; 10 min). Cellular supernatants and culture media were immunoprecipitated with the HCL polyclonal antibody (1:1000 dilution), analyzed by 10% SDS-polyacrylamide gel electrophoresis (PAGE); and submitted to autoradiography.

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The standard curve was constructed with purified human angiotensinogen (Calbiochem).

Renin Measurements—Plasma active renin was measured by IRMA (26) using a commercially available kit (ERIA, Diagnostics Pasteur, Marnes-la-Coquette, France). Plasma renin activity was measured at pH 5.7 according to Sealey et al. (27). The Ang I RIA was performed using the same polyclonal antibody as that used for the angiotensinogen enzymatic assay.
Enzymatic Deglycosylation of Recombinant Angiotensinogen

Deglycosylation of recombinant proteins was performed on immuno-precipitated material (32) that was divided into five aliquots for the following experiments: 1) mock treatment, 2) endoglycosidase H (5 milliunits for 18 h at 37 °C), 3) N-glycosidase F (1 unit for 18 h at 37 °C), 4) N-glycosidase F followed by neuraminidase (5 milliunits for 18 h at 37 °C and pH 6), and 5) the same as aliquot 4 followed by O-glycosidase (2.5 milliunits for 17 h at 37 °C).

RESULTS

Plasma Angiotensinogen Levels in Heterozygous Y248C Individuals—Among the nine analyzed individuals, five were heterozygous (Y248C), all belonging to the maternal branch of the family (Fig. 1). For plasma angiotensinogen levels, all individuals were considered, except subjects I-1 (86 years, treated with thiazides) and III-2 (oral contraceptive). The individuals not bearing the mutation (Y248Y) had significantly higher plasma angiotensinogen concentrations compared with the heterozygous individuals (Y248Y (n = 3): 1915 ng of Ang I/ml, range of 1270–2388 versus Y248C (n = 4): 1100 ng of Ang I/ml, range of 854–1316, p < 0.03) (Table I). The consequence of this difference in angiotensinogen levels on Ang I production can be estimated by the ratio between plasma renin activity and renin concentration (active renin), which represents the amount of Ang I that can be generated in vitro for a constant renin concentration (Ang I production rate). In this pedigree, Y248C subjects present a 35% decrease in Ang I production rate (Y248Y (n = 3): 74.0 pg of Ang I/h, range of 54.2–96.0 versus Y248C (n = 4): 48.3 pg of Ang I/h, range of 42.7–55.7, p < 0.02).

The frequent Thr-235 allele has been previously associated with a 10–20% increase in plasma angiotensinogen concentration (11). In the present pedigree, seven subjects were found to be M235T heterozygotes, originating from both branches of the pedigree (Fig. 1). When a comparison was made between M235T and M235M individuals, independently of the presence of the Cys-248 mutation, no relationship could be established with plasma angiotensinogen levels (Table I). The presence of the Cys-248 mutation in the heterozygous state was associated by itself with a decrease in both plasma angiotensinogen and Ang I production rate.

In Vitro Altered Immunological Recognition of the Cys-248 Variant—Angiotensinogen concentrations measured by enzymatic assay were compared with the values obtained using direct RIA with the polyclonal antibody HCL. These measurements were performed for recombinant wild-type and mutant angiotensinogens secreted in serum-free medium from 9 Tyr-248 and 10 Cys-248 CHO cell clones. Both assays were highly correlated (r = 0.982 for Tyr-248 and r = 0.955 for Cys-248), but the slopes of the correlations obtained for Tyr-248 and Cys-248 angiotensinogens were significantly different (Fig. 2A). For the same level of angiotensinogen measured by enzy-
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To document further the altered immunological recognition of the Cys-248 variant, measurements of both proteins were performed with the angiotensinogen RIA and IRMA using different mAbs. The ratio between the RIA and the enzymatic assay showed important differences according to the mAbs used. IRMA with either the 7C11 or 1C11 mAb as the labeled antibody showed a strong discrimination between the wild-type and mutant proteins (Fig. 2B). The ratio of the angiotensinogen concentration measured by the 1H8–1C11 and 7B2–4G3 antibody pairs was 1.35 ± 0.11 for Tyr-248 and 0.18 ± 0.03 for Cys-248 (7.5-fold difference) (Fig. 3A), whereas ratios between other pairs did not significantly differ (data not shown).

Cys-248 Angiotensinogen Is Present in Plasma of Heterozygous Individuals—The peculiar immunological recognition of Cys-248 angiotensinogen by some monoclonal antibodies allowed us to detect Cys-248 angiotensinogen in plasma of heterozygous Y248C individuals. RIA and IRMA using the most discriminating mAb pairs, cited above, were performed in duplicate and in two separate experiments for each plasma sample. As for recombinant angiotensinogen produced in vitro, the ratio between IRMAs using 1H8–1C11 and 7B2–4G3 was calculated. Expressing results as an immunological ratio has the advantage of being independent of the level of angiotensinogen itself and is only sensitive to the proportion of the wild-type and mutant proteins in each individual. Since the frequent M235T molecular variant also alters the immunological recognition of angiotensinogen by these monoclonal antibodies, the comparisons were made separately for M235M and M235T individuals.

Ratios observed in five unrelated M235M control subjects not bearing the Cys-248 variant (1.58 ± 0.06) were on average 73% higher than those observed in the II-2 and II-4 heterozygous Y248C subjects (Fig. 3B). Values obtained in five controls (0.95 ± 0.03) and in the II-1, II-5, II-6, and III-2 Y248Y subjects (0.85, range 0.81–0.88), but 290% higher than those observed in the I-1, II-3, and II-1 Y248C subjects (0.33, 0.30, and 0.31, respectively; p < 0.01), heterozygous for both M235T and Y248C mutations. Taking into account the difference in the IRMA ratio observed for recombinant Tyr-248 and Cys-248 angiotensinogens, these results strongly suggest that Cys-248 angio-
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The Cys-248 Mutation Leads to an Abnormal Glycosylation of the Protein—CHO cell clones expressing Tyr-248 or Cys-248 angiotensinogens were metabolically labeled and, and the expressed angiotensinogens were immunoprecipitated using the polyclonal antibody HCL. Analysis by SDS-PAGE of immunoprecipitated cellular material obtained from Tyr-248 CHO cells showed two specific bands of 55.5 and 58 kDa, as reported previously (33). An additional 61-kDa band was observed for Cys-248 (Fig. 5). Its presence was confirmed in different additional CHO clones and in cellular material obtained after transient transfection into COS cells (data not shown). To test 1) if this additional band contained some carbohydrate residues and 2) if it was secreted in the extracellular medium, various glycosidases treatments were applied to the cellular content (Fig. 6A) and to the secreted material (Fig. 6B) from Tyr-248 and Cys-248 CHO clones. Intracellular recombinant angiotensinogen treated with N-glycosidase F showed a reduced heterogeneity: both mutant and wild-type proteins exhibited two identical bands of 50 and 51.5 kDa (Fig. 6A); and treatment with endoglycosidase H produced a unique band of 50 kDa. The additional 61-kDa band observed for the Cys-248 mutant was sensitive to these two glycosidases and was no longer distinguishable after these enzymatic digestions, indicating that this angiotensinogen species is a particular high mannose immature glycosylated form. As expected, both secreted forms of angiotensinogen were not sensitive to endoglycosidase H, whereas endoglycosidase F resolved both angiotensinogen forms into their respective nonglycosylated species of 50 and 51.5 kDa (Fig. 6B). Finally, treatment of Tyr-248 and Cys-248 angiotensinogens with neuraminidase and O-glycosidase after treatment with N-glycosidase did not significantly change the molecular mass of the proteins, confirming that angiotensinogen glycosylation is predominantly an N-linked glycosylation.

The Cys-248 Mutation Is Associated with an Intracellular Retention of the Protein—The dynamics of the intracellular synthesis and secretion of Tyr-248 and Cys-248 angiotensinogens in CHO cells were compared by pulse-chase experiments. Both wild-type and mutant angiotensinogens were secreted in the medium 20 min after the pulse. Intracellular Tyr-248 angiotensinogen was present in plasma of Y248C individuals of our family.

Immunopurification and Kinetic Parameters of Recombinant Angiotensinogen—Western blot analysis of recombinant Tyr-248 and Cys-248 angiotensinogens secreted by CHO cells gave similar patterns (Fig. 4A). Both proteins were purified by an immunoaffinity column using the angiotensinogen 181 monoclonal antibody. Superimposable elution profiles (Fig. 4B) were observed, suggesting a similar affinity for this monoclonal antibody. Coomassie Blue staining and Western blot analysis of immunopurified Tyr-248 and Cys-248 angiotensinogens. Each protein (2 μg) was denatured with 50 mM dithiothreitol. Proteins were separated on an SDS-9% acrylamide gel and visualized by Coomassie Blue staining. D, Western blot analysis of immunopurified Tyr-248 and Cys-248 angiotensinogens. Each protein (10 ng) was denatured with 10 mM dithiothreitol. Western blot analysis was performed as indicated above.

![Figure 4](http://www.jbc.org/content/early/2018/07/18/jbc.K18.954931F4)

**Characterization and immunopurification of recombinant Tyr-248 and Cys-248 angiotensinogens.** A, Western blot analysis of Tyr-248 and Cys-248 angiotensinogens secreted by CHO cells. Samples were denatured with reducing agent (5 mM dithiothreitol). Western blotting was performed using an anti-angiotensinogen polyclonal antibody HCL (22). Antigen-antibody complexes were revealed by alkaline phosphatase activity after biotin-streptavidin enhancement. B, elution profiles of Tyr-248 and Cys-248 angiotensinogens. CHO cell media containing 196 μg of Tyr-248 protein or 290 μg of Cys-248 protein were loaded onto mAb 181-Sepharose. Bound proteins were eluted with 0.1 M sodium acetate buffer, pH 3.5. The angiotensinogen amount was measured in each fraction by angiotensinogen assays. C, Coomassie Blue staining of immunopurified Tyr-248 and Cys-248 angiotensinogens. Each protein (2 μg) was denatured with 50 mM dithiothreitol. Proteins were separated on an SDS-9% acrylamide gel and visualized by Coomassie Blue staining. D, Western blot analysis of immunopurified Tyr-248 and Cys-248 angiotensinogens. Each protein (10 ng) was denatured with 10 mM dithiothreitol. Western blot analysis was performed as indicated above.
angiotensinogen decreased progressively and was almost completely secreted after 3.5 h (Fig. 7). In contrast, Cys-248 angiotensinogen was secreted more slowly, being clearly present in the cell at 3.5 h and still observed at 48 h. Comparison between the wild-type and mutant patterns showed that this slower processing was mainly due to the 58- and 61-kDa forms, while a similar kinetics of disappearance was observed for the 55.5-kDa form. This intracellular retention of Cys-248 angiotensinogen was confirmed using different chase periods and with several CHO clones.

**DISCUSSION**

The presence of natural mutations of the AGT gene provides a unique opportunity to obtain further insight into the pathophysiological impact of angiotensinogen on the renin-angiotensin system and blood pressure regulation. Among the different genetic variations discovered in the coding segment of the AGT gene, the change from Tyr to Cys at position 248 of angiotensinogen could represent a mutation with a negative impact on the renin system activity. In the family studied here, the genetic variations discovered in the coding sequence of the AGT gene (8). However, pathophysiological adaptations of other systems regulating blood pressure and especially feedback regulation by renin should play a major role in the final blood pressure level (34, 35).

To investigate the mechanism responsible for this reduction of angiotensinogen concentration in Y248C individuals, several authors demonstrated a heterogeneity of the protein varying from 55 to 65 kDa, which corresponds mainly to multiple glycosylation sites (Asn-X-Ser/Thr) that represent 14% of the molecular mass of the protein (1, 2). Using different multiple-step procedures for the purification of angiotensinogen, several authors demonstrated a heterogeneity of the protein varying from 55 to 65 kDa, which corresponds mainly to multiple glycosylation sites (Asn-X-Ser/Thr) that represent 14% of the molecular mass of the protein (1, 2). For both wild-type and mutant proteins, a similar heterogeneous pattern and molecular mass were identified by Western blot analysis (Fig. 4A) and by metabolic labeling (Fig. 5A) in CHO cell medium. However, several lines of evidence suggest structural differences between Tyr-248 and Cys-248 angiotensinogens. After immunopurification, Cys-248 angiotensinogen displayed a slower migration on SDS-PAGE (-3 kDa) as compared with Tyr-248 angiotensinogen (Fig. 4, C and D), indicating that the acidic denaturation and renaturation used during the immunopurification procedure modified the two angiotensinogens differently. In addition, we were able to demonstrate that the Tyr to Cys change at position 248 of angiotensinogen negatively affects the immunological recognition of the protein. Among the four monoclonal antibodies directed against human angiotensinogen that had been previously generated (23), two (4G3 and 1C11) that belong to the same antigenic cluster2 allowed a clear distinction between the mutant and wild-type forms of the protein and the detection of Cys-248 angiotensinogen in plasma of Y248C individuals.

Whether the altered immunological recognition of Cys-248 angiotensinogen is due to the modification of the carbohydrate content or to a more profound structural modification due to the change of amino acids cannot be determined from this study. It is interesting to note that the Tyr residue at position 248 is part of a region of six residues that are conserved...
between proteins of the serine protease inhibitor family (37, 38). From the three-dimensional structures of α1-antitrypsin and antithrombin III, this conserved sequence corresponds to the s3 segment of the large β-sheet A, which is not exposed at the surface of the molecule. Since comparison between wild-type and Cys-248 angiotensinogens shows a significant alteration of the predicted hydrophy profile (39), it is tempting to speculate that the mutation disrupts the association with the following antiparallel strand of the β-sheet A, thus allowing the Cys residue at position 248 to be exposed at the surface and to modify the corresponding epitope site of angiotensinogen. However, this interpretation should be cautiously accepted since the entire α1-antitrypsin and antithrombin III molecules share only 21 and 18% similarity at the amino acid level with angiotensinogen. A potential impact of these structural modifications either on plasma angiotensinogen half-life or on its interaction with other plasma proteins (40) was not tested in this study. However, we did not observe any modification of the functional properties of the protein as a renin substrate, but only a decrease in intracellular processing of the mutant.

The other major difference between Cys-248 and Tyr-248 angiotensinogens was observed during the biosynthesis and secretion of the corresponding recombinant proteins. In all cases analyzed, the Cys-248 mutant presented a 61-kDa intracellular form in addition to the two bands of 55.5 and 58 kDa observed with the wild-type protein. Since treatment with endoglycosidase H or F suppressed this difference, it was interpreted as a modification in the glycosylation process. In addition to this qualitative change of glycosylation, pulse-chase studies demonstrated that the intracellular processing of the different forms of angiotensinogen was not similar. While the intracellular 55.5-kDa form was similarly secreted for both proteins, the 58- and 61-kDa forms of Cys-248 angiotensinogen were significantly retained in CHO cells, suggesting an abnormal intracellular processing of the mutant protein.

These experimental observations may be differently interpreted. One explanation is that the structural modifications induced by the Cys-248 mutation markedly affect the processing of the protein, explaining the accumulation of different high mannose forms of angiotensinogen. Therefore, the observed 61-kDa band could represent an initial transient and rapidly processed glycosylated form of angiotensinogen synthesized for both the Tyr-248 and Cys-248 proteins, which is not detectable unless a slower intracellular processing occurs. Another more speculative but also attractive hypothesis is that the change from Tyw to Cys at residue 248 creates an unusual site of N-glycosylation (Asn-X-Cys) (41). According to this hypothesis, the 61-kDa form of Cys-248 angiotensinogen would present an additional N-linked carbohydrate side chain that would be deleterious for the efficient secretion of angiotensinogen. Indeed, N-glycans play a major role in protein folding and intracellular processing of secretory protein (42). Both hypotheses could explain the abnormal glycosylation and secretion that were observed in CHO cells, but cannot be directly extrapolated to other cellular systems. In both cases, the observed decreased plasma angiotensinogen concentration in subjects bearing the Cys-248 mutation may be explained by altered intracellular processing and secretion of angiotensinogen in hepatocytes.

In conclusion, this study demonstrates that the presence of the rare Cys-248 variant in the heterozygous state is associated with a significantly lower plasma angiotensinogen concentration. Recombinant Cys-248 angiotensinogen exhibits an altered immunological profile and is abnormally glycosylated and secreted in CHO cells. Taken together, these results strongly suggest that the Cys-248 variant of angiotensinogen alters the structure of the protein in addition to decreasing its concentration in individuals heterozygous for this mutation.

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