Corin Mutation R539C from Hypertensive Patients Impairs Zymogen Activation and Generates an Inactive Alternative Ectodomain Fragment*

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Background: Corin is a membrane serine protease that activates natriuretic peptides in the heart.
Results: The corin mutant R539C identified in hypertensive patients has impaired zymogen activation and altered ectodomain shedding.
Conclusion: The mutation alters corin protein structure and reduces corin activity.
Significance: Human *CORIN* gene mutations causing impaired corin activity may be an underlying mechanism in hypertension.

Corin is a cardiac transmembrane serine protease that regulates blood pressure by activating natriuretic peptides. Corin variants have been associated with African Americans with hypertension and heart disease. Here, we report a new mutation in exon 12 of the *CORIN* gene identified in a family of patients with hypertension. The mutation resulted in R539C substitution in the Fz2 (Frizzled-2) domain of the corin propeptide region. We expressed and characterized the corin R539C mutant in HEK293 cells. As determined by Western blot analysis, the R539C mutation did not alter corin expression in transfected cells but impaired corin zymogen activation. In a pro-atrial natriuretic peptide processing assay, the corin mutant had reduced activity and exhibited a dominant-negative effect on wild-type corin. In addition, the R539C mutation altered corin ectodomain shedding, producing an alternative ~75-kDa fragment that was biologically inactive. Using protease inhibitors and the catalytically inactive corin mutant S985A, we showed that the ~75-kDa fragment was generated by corin autocleavage. We constructed a series of mutants by replacing single or double Arg residues in the corin propeptide and identified Arg-530 in the Fz2 domain as the alternative autocleavage site. Our results show that the corin mutation R539C identified in hypertensive patients impairs corin zymogen activation and causes an alternative autocleavage that reduces corin activity. These data support that human *CORIN* gene muta-
tions causing impaired corin activity may be an underlying mechanism in hypertension.

Hypertension is a common cardiovascular disease, afflicting nearly 30% adults in developed countries (1). Genetic factors are known to influence the onset and severity of the disease. Mutations in the genes involving electrolyte homeostasis have been reported in hypertensive patients (2–5). To date, however, the underlying disease mechanism in the majority of patients with hypertension remains unknown (4).

Atrial natriuretic peptide (ANP)[3] plays an important role in regulating salt-water balance and blood pressure (6, 7). Genetic variants or mutations of the genes encoding ANP or its receptor have been associated with hypertension in patients (8–11). Corin is a transmembrane serine protease that converts the ANP precursor (pro-ANP) to mature ANP in cardiac myocytes (6, 12). In mice, lack of corin impairs renal sodium excretion and causes salt-sensitive hypertension and cardiac hypertrophy (13–16). These data support the physiological importance of corin and ANP in maintaining normal blood pressure.

Structurally, corin belongs to the family of type II transmembrane serine proteases that participate in diverse biological processes (17). Mutations in genes encoding these proteases have been reported to cause human diseases. For example, mutations in the *TMPRSS3*, *TMPRSS6*, and *ST14* genes have been identified in patients with congenital deafness, iron-refractory anemia, and ichthyotic skin disorders, respectively (18–25). The human *CORIN* gene is located on chromosome 4, which contains 22 exons and spans >200 kb in length (26). *CORIN* gene variants (T555I/Q568P) have been identified in African Americans with hypertension and heart disease (27, 28). Studies have shown that the variants have reduced natriuretic peptide processing activity *in vitro* and *in vivo* (29, 30). Most

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[3] The abbreviations used are: ANP, atrial natriuretic peptide; LDLR, LDL receptor.

recently, two *CORIN* gene mutations have been reported in patients with pregnancy-induced hypertension (31). The data suggest that variants or mutations of the *CORIN* gene may contribute to hypertensive disease in humans.

To test this hypothesis, we sequenced the *CORIN* gene in patients with hypertension. Here, we report a novel *CORIN* gene mutation (R539C) identified in a patient family with hypertension. We characterized the corin mutant R539C in a series of biochemical and cellular experiments to understand how the mutation may alter corin protein structure and function.

EXPERIMENTAL PROCEDURES

Patient Blood Samples—Venous blood samples were from 183 patients with hypertension that was defined by systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg. Additional blood samples were obtained from 229 normal individuals who underwent routine checkups at the hospitals and had no history of hypertension. All individuals were ethnic Han Chinese. The study was approved by the ethic committees at the hospitals. All participants provided written informed consent.

DNA Extraction and Sequencing—Genomic DNA was extracted from peripheral blood cells using the QIAamp DNA blood kit (Qiagen). DNA fragments from all 22 exons and intron-exon boundaries of the *CORIN* gene were amplified by PCR using specific oligonucleotide primers. PCR products were sequenced directly by an Applied Biosystems 3100 DNA analyzer. Gene variants or mutations identified were verified by independent PCR and DNA sequencing.

Plasmids—Plasmids expressing human WT corin, the S985A mutant (in which the catalytic Ser-985 was replaced with Ala), and the R801A mutant (in which the activation cleavage site Arg-801 was replaced with Ala) were described previously (32, 33). Plasmids expressing corin mutants R539C, R539A, R539C/R480A, R530A/R539C, R539C/R588A, R539C/R597A/R598A, R539C/R619A, R539C/R670A, R539C/S985A, C540A, and R539C/C540A were made by PCR-based site-directed mutagenesis. Recombinant corin proteins encoded by these plasmids contained a C-terminal V5 tag to facilitate protein detection by Western blotting (32, 33).

Transfection, Immunoprecipitation, and Western Blotting—HEK293 cells were grown in DMEM with 10% FBS in humidified incubators at 37 °C with 5% CO_2 and 95% air. Plasmids were transfected into HEK293 cells using FuGENE reagents (Roche Diagnostics). Conditioned medium was collected after 48–72 h. Cells were lysed in buffer containing 50 mmol/liter Tris-HCl (pH 8.0), 150 mmol/liter NaCl, 1% (v/v) Triton X-100, and a protease inhibitor mixture (1:100 dilution; Sigma). Immunoprecipitation and Western analysis of corin proteins were done using an anti-V5 tag antibody as described previously (34, 35).

Pro-ANP Processing Assay—Conditioned medium containing recombinant human pro-ANP from a stable HEK293 cell line was added to HEK293 cells expressing WT corin or mutants and incubated at 37 °C for 2 h. Pro-ANP and ANP in the medium were analyzed by immunoprecipitation and Western blotting (34, 35). To test the activity of soluble corin, conditioned medium containing corin fragments from transfected HEK293 cells was collected, concentrated, and incubated with the pro-ANP medium at 37 °C for 2 h. Pro-ANP processing was assessed by immunoprecipitation and Western blotting.

Effect of Protease Inhibitors on Corin Cleavage—To identify proteases involved in corin cleavage, a panel of protease inhibitors, including benzamidine (a trypsin-like serine protease inhibitor; 5 mM), GM6001 (a metalloproteinase inhibitor; 50 μM), TAPI-1 (an ADAM (a disintegrin and metalloproteinase) inhibitor; 50 μM), and *N*-acetyl-leucyl-leucyl-methionine (a cysteine protease inhibitor; 50 μM), was added to corin-expressing cells in separate wells of culture plates. The conditioned medium was collected over time. Corin fragments in the conditioned medium were analyzed by immunoprecipitation and Western blotting.

Molecular Modeling—Modeling of the corin Fz2 (Frizzled-2) domain was done based on the crystal structure of mouse Frizzled-8 (36). The sequences of mouse Frizzled-8 and the human corin Fz2 domain were aligned using the ClustalW program (37). Three-dimensional models of the human corin Fz2 domain were created using a computer-based homology modeling program (38). The model inspection and image generation were carried out using the PyMOL program.

Statistical Analysis—Data were analyzed using SPSS 12 software (SPSS, Chicago, IL). All data are presented as means ± S.D. Comparisons between two groups were done by Student's *t* test. Multigroup comparisons were done by analysis of variance. A *p* value of <0.05 was considered to be statistically significant.

RESULTS

Identification of CORIN Gene Mutation—We sequenced the *CORIN* gene from 183 patients with hypertension. A C → T heterozygous mutation at nucleotide 1708 in exon 12 was identified in a hypertensive patient (Fig. 1, *A* and *B*). This mutation was absent in 229 unrelated healthy controls. Members of the patient family who carried this mutation had higher blood pressure except for a 14-year-old male (IV-3) (Fig. 1*A*). The mutation caused an Arg-to-Cys substitution at residue 539 in the Fz2 domain (Fig. 2*A*). In a three-dimensional model of the corin Fz2 domain (Fig. 2, *B* and *C*), Arg-539 was located in an α-helix on the domain surface. The model also suggested that the mutant Cys residue was likely to create an alternative disulfide bond with Cys-502 in an adjacent loop (Fig. 2*C*), which may alter the overall Fz2 domain structure.

Expression and Functional Analysis of the R539C Mutant—To examine if the R539C mutation altered corin expression and/or function, we made plasmids expressing the R539C and R539A mutants and transfected them into HEK293 cells. WT corin and the activation cleavage site mutant R801A and the catalytic active site mutant S985A were used as controls. On Western blots, WT and mutant corin proteins were found to be expressed at similar levels in transfected cells (Fig. 3*A*). However, the level of the ~40-kDa band in the R539C mutant was lower than those in WT corin and mutants R539A and S985A. This band represents the corin protease domain cleaved at the activation site Arg-801 (Fig. 2*A*). As predicted, the band was absent in the R801A mutant, which abolished the activation
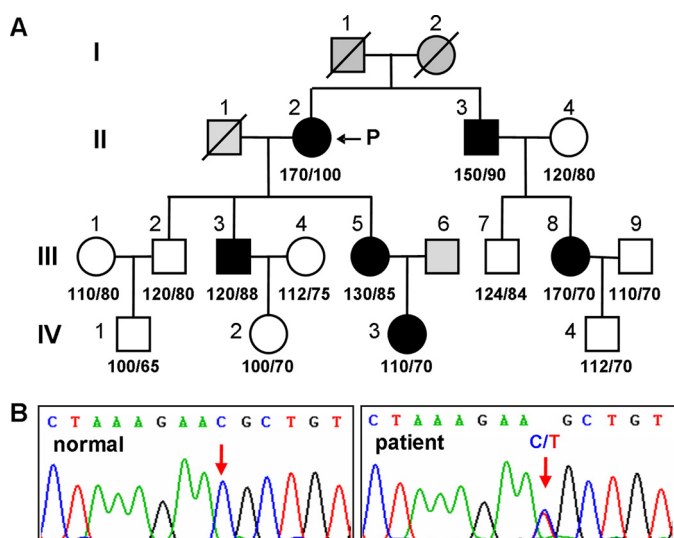
FIGURE 1. **CORIN gene mutation in a patient family.** *A*, patient pedigree. Males (*squares*) and females (*circles*) with (*black*) or without (*white*) the R539C mutant allele are shown. The *arrow* indicates the proband (*P*). *Slashes* through the symbols indicate decreased individuals. *Gray* indicates the individuals from whom samples were not available. *Numbers* below the symbols indicate systolic/diastolic blood pressure (mm Hg) in these individuals. *B*, *CORIN* gene sequencing data from a normal control (*left panel*) and the proband (*right panel*). The C → T heterozygous mutation at nucleotide 1708 causing the R539C change is indicated (*right panel*).
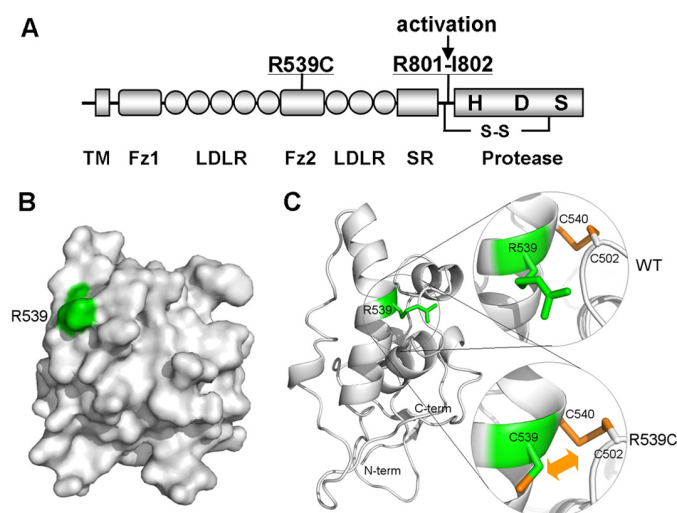

FIGURE 2. **Location of the R539C mutation in the corin Fz2 domain.** *A*, corin protein domains. *TM*, transmembrane; *SR*, scavenger receptor. The catalytic residues His (*H*), Asp (*D*), and Ser (*S*) are shown. The *arrow* indicates the activation cleavage site at Arg-801–Ile-802. A disulfide bond (*S-S*) links the propeptide and the protease domain. *B*, a surface model of the corin Fz2 domain by molecular modeling. Arg-539 is in *green*. *C*, location of Arg-539 in a molecular model of the corin Fz2 domain. The R539C mutation may potentially create an alternative disulfide bond between Cys-539 and Cys-502. Magnified areas highlight the residues involved in the WT corin (*upper circle*) and R539C mutant (*lower circle*) models. The N (*N-term*) and C (*C-term*) termini of the Fz2 domain are indicated. The conserved disulfide bond between Cys-540 and Cys-502 is shown in *stick models*.

cleavage site (Fig. 3*A*). As estimated by densitometric analysis of the Western blots, the levels of the ~40-kDa band were 28.0 ± 10.3 and 17.8 ± 8.3%, respectively, of the total WT and R539C corin proteins ($p < 0.01$) (Fig. 3*B*). The data indicate that the R539C (but not R539A) mutation impairs corin zymogen activation.

We next tested the activity of corin mutants in a pro-ANP processing assay. The activity of the R539C (but not R539A) mutant was significantly reduced compared with that of WT corin (Fig. 3*C*). Two negative controls (mutants R801A and S985A) had little activity (Fig. 3*C*). As estimated by densitometric analysis of the Western blots, the R539C mutant had 59.8 ± 16.0% activity compared with WT corin ($p < 0.01$) (Fig. 3*D*).

Based on the molecular model (Fig. 2*C*), R539C is likely to interfere with the disulfide bond between Cys-540 and Cys-502. Consistent with this hypothesis, mutant C540A, which was expected to disrupt the disulfide bond, had markedly reduced pro-ANP processing activity (26.7 ± 5.5% of WT corin, $p < 0.01$) (Fig. 3, *E* and *F*). When both the R539C and C540A mutations were introduced, the activity of mutant R539C/C540A was significantly higher than that of mutant C540A (55.3 ± 3.2 *versus* 26.7 ± 5.5%, $p < 0.01$) (Fig. 3, *E* and *F*), suggesting that the mutant Cys-539 residue may pair with Cys-502 in the absence of Cys-540 (Fig. 2*C*), thereby partially restoring the corin structure and activity.

Cleaved Corin Fragments in Conditioned Medium—Previous studies showed that corin is shed from the cell surface, producing three major soluble fragments of ~180, ~160, and ~100 kDa (Fig. 4*A*) (32). To examine if the R539C mutation affects corin shedding, we analyzed corin fragments in the conditioned medium from transfected HEK293 cells. Three bands of ~180, ~160, and ~100 kDa were detected in the conditioned medium from HEK293 cells expressing WT corin (Fig. 4*B*, *upper panel*). The sample from the inactive corin mutant R801A had only the ~180-kDa band, consistent with previous findings that the ~160- and ~100-kDa bands are products of corin autocleavage (32). In the sample from the R539C mutant, a prominent extra band of ~75 kDa was detected (Fig. 4*B*, *upper panel*). This band also was observed in the sample from the C540A mutant, but not in the samples from mutants R539A and R539C/C540A (Fig. 4*B*, *upper panel*).

In a time course experiment with the conditioned medium collected over time from HEK293 cells expressing the R539C mutant, the ~180-kDa band appeared in samples after 16 h, whereas the ~75-kDa band appeared only in samples after 40 h (Fig. 4*C*). To examine if the ~75-kDa band was derived from the ~180-kDa fragment, the conditioned medium was collected at 24 h after transfection and incubated at 37 °C over time without the cells. Western analysis showed the ~75-kDa fragment in the sample incubated for 48 h (Fig. 4*D*). In the same sample, the level of the ~180-kDa fragment was reduced, indicating that the ~75-kDa fragment was derived from the ~180-kDa fragment in this experiment.

Effect of Protease Inhibitors on Corin Cleavage—To determine the class of enzyme(s) responsible for generating the ~75-kDa fragment, we added protease inhibitors to HEK293 cells expressing the R539C mutant and analyzed the conditioned medium by Western blotting. The level of the ~75-kDa band was markedly reduced in the presence of benzamidine, but not metalloproteinase (GM6001 and TAPI-1) or cysteine protease (*N*-acetyl-leucyl-leucyl-methionine) inhibitors (Fig. 5*A*), indicating that the ~75-kDa fragment was likely generated by a serine protease.
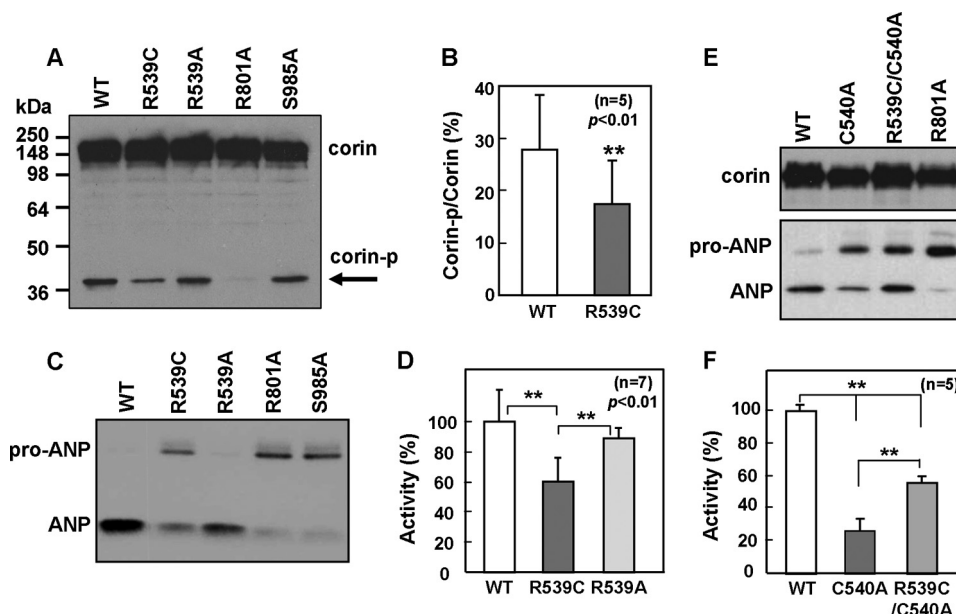
FIGURE 3. **R539C mutation reduces zymogen activation and pro-ANP processing activity.** *A*, Western analysis of lysates from HEK293 cells expressing WT corin and mutants. The cleaved corin protease domain fragment (*corin-p*) is indicated by the *arrow*. *B*, percentage of the activated protease fragment *versus* corin zymogen protein as estimated by densitometry. Data are means ± S.D. from five independent experiments. *C*, Western analysis of pro-ANP processing activities of corin mutants. *D*, quantitative data of pro-ANP processing as estimated by densitometry. Data are means ± S.D. from seven independent experiments. **, $p < 0.01$ *versus* WT corin or R539A. *E*, protein expression (*upper panel*) and pro-ANP processing activity (*lower panel*) of corin mutants C540A and R539C/C540A. WT corin and mutant R801A were used as positive and negative controls, respectively. *F*, quantitative data (means ± S.D., $n = 5$) of pro-ANP processing by WT corin and mutants C540A and R539C/C540A. **, $p < 0.01$ *versus* the indicated groups.
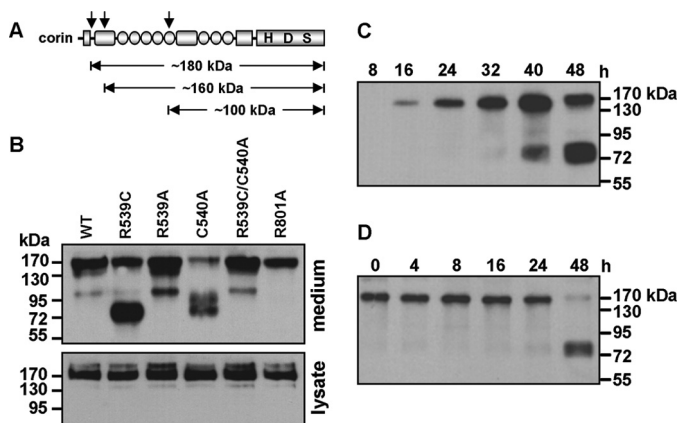

FIGURE 4. **Cleaved corin fragments in conditioned medium.** *A*, illustration of corin fragments. Cleavage sites generating three soluble fragments are indicated by *arrows*. *B*, Western analysis of corin fragments in the conditioned medium from cells expressing WT corin and mutants (*upper panel*). Corin proteins in cell lysate are shown as a control (*lower panel*). *C*, Western analysis of corin fragments in the conditioned medium collected over the indicated time periods from cells expressing the R539C mutant. *D*, Western analysis of corin fragments in the conditioned medium collected at 24 h after transfection from cells expressing the R539C mutant and then incubated at 37 °C for the indicated time periods without the cells.
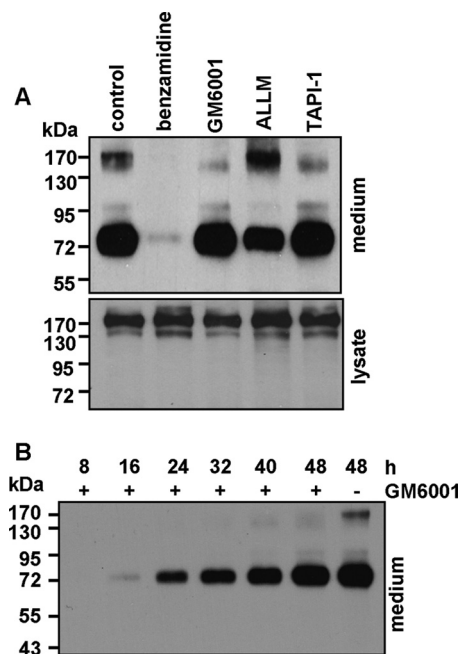

FIGURE 5. **Effect of protease inhibitors.** *A*, HEK293 cells expressing the R539C mutant were incubated with vehicle (*control*), benzamidine, GM6001, *N*-acetyl-leucyl-leucyl-methionine (*ALLM*), or TAPI-1. Corin fragments in the conditioned medium were analyzed by Western blotting (*upper panel*). Corin proteins in cell lysate are shown as a control (*lower panel*). Data are representative of three independent experiments. *B*, Western analysis of corin fragments in the conditioned medium collected over the indicated time periods from cells expressing the R539C mutant with (+) or without (−) GM6001.

As shown in Fig. 5*A*, GM6001 and TAPI-1 reduced the level of the ~180-kDa fragment, consistent with a previous report that the fragment was cleaved by an ADAM protease (32). The results also indicated that the production of the ~75-kDa fragment may not depend on the ~180-kDa fragment. To verify this hypothesis, we added GM6001 to HEK293 cells expressing the R539C mutant and collected the conditioned medium over time. GM6001 inhibited the production of the ~180-kDa fragment, but not the ~75-kDa fragment (Fig. 5*B*), indicating that

the presence of the ~180-kDa fragment was not a prerequisite for generating the ~75-kDa fragment.

Biological Activity of Soluble Corin Fragments—To test whether the ~75-kDa fragment is biologically active, we incu-
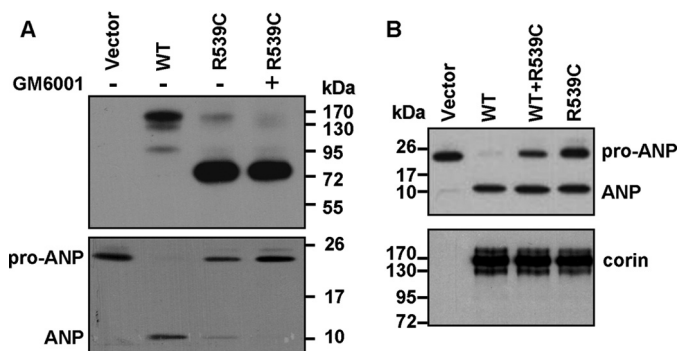
FIGURE 6. **Activity of soluble corin fragments and effect of R539C on WT corin activity.** *A*, cells expressing WT corin or mutants were cultured without (−) or with (+) GM6001. Corin fragments in the conditioned medium (*upper panel*) and their pro-ANP processing activities (*lower panel*) were analyzed by Western analysis. Data are representative of three independent experiments. *B*, HEK293 cells were transfected with plasmids expressing WT corin and R539C either alone or together. Corin activity was measured in a pro-ANP processing assay by Western blotting (*upper panel*). Corin proteins in cell lysate are shown as a control (*lower panel*). Data are representative of five independent experiments.

bated the conditioned medium with recombinant human pro-ANP and analyzed pro-ANP processing. Pro-ANP processing activity was detected in the conditioned medium from cells expressing WT corin (Fig. 6*A*). The activity was markedly lower or absent in the conditioned medium from R539C mutant-expressing cells cultured without or with GM6001, which inhibited the ~180-kDa fragment (Fig. 6*A*). The results indicated that the ~75-kDa fragment had little biological activity.

We also tested the effect of the R539C mutant on WT corin in a pro-ANP processing assay. In HEK293 cells cotransfected with plasmids expressing WT corin and the R539C mutant, pro-ANP processing activity was significantly reduced (64.4 ± 5.3% of WT corin, $p < 0.01$) (Fig. 6*B*, *upper panel*), indicating a dominant-negative effect of the mutant on WT corin.

Corin Autocleavage—The inhibition of the ~75-kDa fragment by benzamidine indicated that the fragment was derived from serine protease-mediated cleavage (Fig. 5*A*). Corin is a serine protease, capable of generating the ~160- and ~100-kDa fragments by autocleavage (32). To test whether the ~75-kDa fragment was also from autocleavage, we made a double mutant (R539C/S985A) that was catalytically inactive (Fig. 7*A*). Western analysis did not detect the ~75-kDa band in the conditioned medium from cells expressing the R539C/S985A mutant (Fig. 7*B*), confirming that the fragment was generated by corin autocleavage.

Identification of Alternative Corin Autocleavage Site—Based on the size of the ~75-kDa fragment, the cleavage site for this fragment was predicted to be in a region including the Fz2 domain and LDL receptor (LDLR) 6–8 repeats (Fig. 7*A*). To identify the specific cleavage site, we made a series of corin mutants by replacing single or double Arg residues with Ala within this region: R480A, R530A, R588A, R597A/R598A, R619A, and R670A (Fig. 7*A*). Western analysis of the conditioned medium from cells expressing these mutants showed that the R530A mutation, but not the other mutations, abolished the ~75-kDa fragment (Fig. 7*C*). The results showed that the R539C mutant autocleaved at Arg-530 in the Fz2 domain to generate the alternative ~75-kDa fragment.

DISCUSSION

In this study, we have reported a novel mutation (R539C) in the corin Fz2 domain identified in a hypertensive patient family. In functional experiments, the R539C mutation did not alter corin expression in transfected cells but impaired corin zymogen activation, as indicated by reduced amounts of activated protease domain fragment (Fig. 3, *A* and *B*). Consistently, the R539C mutant had reduced activity in processing pro-ANP (Fig. 3, *C* and *D*).

Based on a three-dimensional model of the corin Fz2 domain, Arg-539 is in an α-helix and is spatially close to Cys-502, which forms a disulfide bond with Cys-540 (Fig. 2*C*). The change of Arg-539 to Cys may create an alternative disulfide bond with Cys-502, thereby leaving Cys-540 unpaired and altering corin structure and function. In agreement with this hypothesis, when Cys-539 was substituted with Ala, the corin R539A mutant exhibited similar zymogen activation and activity compared with WT corin (Fig. 3, *A–D*), indicating that Arg at position 539 is not critical but that substitution of this Arg residue with Cys may alter the structure of corin and impair its function. In support of this hypothesis, mutant C540A, which disrupted the predicted disulfide bond between Cys-540 and Cys-520, had markedly reduced activity (Fig. 3, *E* and *F*). This defect was partially corrected when both mutations R539C and C540A were introduced (Fig. 3, *E* and *F*), suggesting that the mutant Cys-539 residue may form a disulfide bond with Cys-502, mimicking the original disulfide bond between Cys-540 and Cys-502 (Fig. 2*C*).

Ectodomain shedding is important in regulating protein expression and activity on the cell surface (39, 40). As a membrane protease, corin is shed by ADAM or autocleaved to generate distinct soluble fragments, which regulates corin activity in cardiac myocytes (32). In this study, we examined if the R539C mutation alters corin ectodomain shedding. Consistent with a previous report (32), three soluble fragments of ~180, ~160, and ~100 kDa were detected in the conditioned medium from cells expressing WT corin. Unexpectedly, a major alternative fragment of ~75 kDa was found in the conditioned medium from cells expressing the R539C (but not R539A) mutant (Figs. 4*B* and 7*B*), suggesting that this fragment was produced as a result of the conformational change caused by the mutant Cys-539 residue. Consistent with this hypothesis, the alternative ~75-kDa fragment also was detected in the sample from mutant C540A (Fig. 4*B*), which was expected to disrupt the disulfide bond between Cys-540 and Cys-502 (Fig. 2*C*). When both mutations R539C and C540A were introduced, the ~75-kDa fragment disappeared (Fig. 4*B*), suggesting that, in the absence of Cys-540, Cys-539 may form a disulfide bond with Cys-502, thereby partially restoring the altered conformational change in the Fz2 domain.

Using protease inhibitors and the catalytically inactive corin mutant S985A (Figs. 5*A* and 7*B*), we showed that the ~75-kDa fragment was generated by corin autocleavage. We also found that this fragment could be generated directly from the membrane-bound corin or from the ~180-kDa soluble fragment as an intermediate (Figs. 4*D* and 5*B*). By testing a series of mutants with replacements of single or double Arg residues in the Fz2
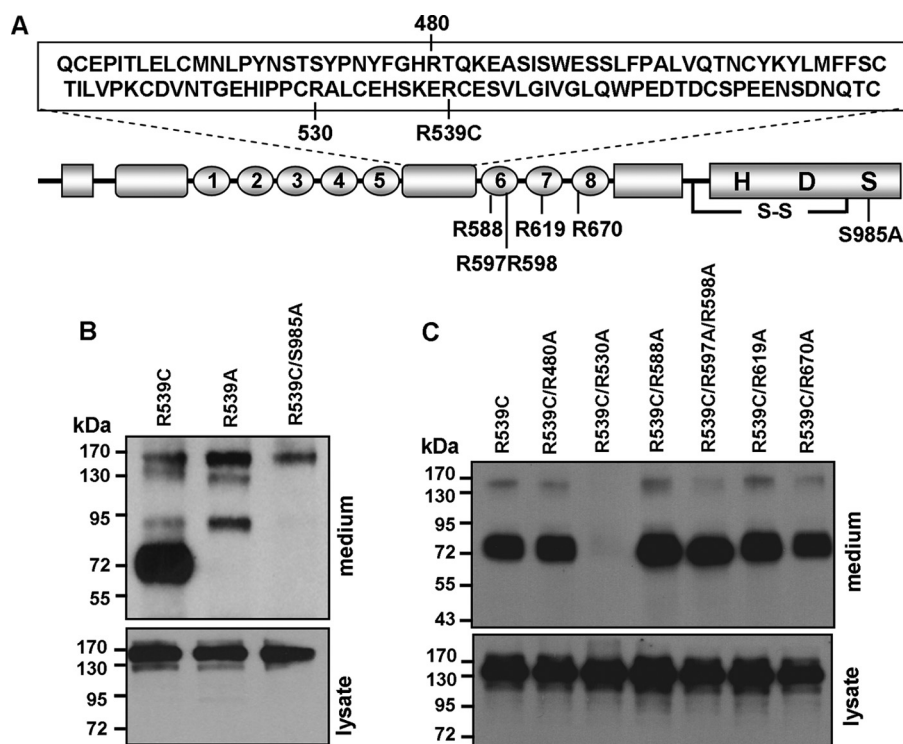
FIGURE 7. **Corin autocleavage sites.** *A,* illustration of Arg residues in the Fz2 and LDLR6 – 8 domains and the active site mutant S985A in the protease domain. *H,* His; *D,* Asp; *S,* Ser; *S-S,* disulfide bond. *B,* Western analysis of corin fragments in the conditioned medium from cells expressing WT corin and mutants (*upper panel*). Corin proteins in cell lysate are shown as a control (*lower panel*). Data are representative of three independent experiments. *C,* Western analysis of corin fragments in the conditioned medium from cells expressing corin mutants (*upper panel*). Corin proteins in cell lysate are shown as a control (*lower panel*).

and LDLR6 – 8 domains, we showed that the ~75-kDa fragment was from specific cleavage at Arg-530 in the Fz2 domain (Fig. 7, *A* and *C*). As indicated in the Fz2 domain model (Fig. 2*C*), Arg-530 is located in the N-terminal region of the α-helix that includes Arg-539 in the middle. It appears that the R539C mutation may cause a mismatched disulfide bond with Cys-502 or possibly Cys-540, resulting in a distorted conformation of the α-helix and creating an alternative autocleavage site at Arg-530.

The biological function of corin is to activate pro-ANP (6, 12). Previously, the ~180-kDa fragment was shown to be generated by ADAM and to be biologically active, whereas the ~160- and ~100-kDa fragments were generated by autocleavage and were inactive (32). In this study, the ~75-kDa fragment from alternative corin autocleavage had little pro-ANP processing activity (Fig. 6*A*). The results are consistent with previous findings that the Fz1 and LDLR1–5 domains, which precede the Fz2 domain (Fig. 2*A*), are required for pro-ANP processing (41). It is interesting that all corin fragments from autocleavage identified so far are biologically inactive, suggesting that autocleavage may serve as a mechanism to negatively regulate corin activity.

Corin is made primarily in cardiac myocytes (42). Cleaved corin fragments may enter the circulation. Corin antigen and activity have been detected in human blood (43– 46). Studies indicate that the levels of plasma soluble corin and activity are significantly lower in patients with hypertension and heart failure (43, 47, 48), suggesting that the reduced corin activity may contribute to hypertensive disease.

In this study, we identified a heterozygous corin mutation in a patient family with hypertension. In cotransfected HEK293 cells, the R539C mutant inhibited WT corin in a pro-ANP processing assay (Fig. 6*B*). The results indicate a possible dominant-negative effect of the mutant protein, which may also occur in the patients. Within this patient family, most members with the mutant allele had higher systolic and/or diastolic blood pressure than those with two normal alleles (Fig. 1*A*). Individual IV-3, a 14-year-old male, appeared to be an exception; he had a mutant allele but normal blood pressure. It is known that the prevalence of hypertension increases with age (49), suggesting that age-related factors play an important role in regulating blood pressure. It would be interesting to know if this individual develops hypertension when he becomes older. Together, our results indicate that the naturally occurring R539C mutation in hypertensive patients may reduce corin activity by impairing zymogen activation and producing an inactive alternative autocleavage fragment. These findings confirm that *CORIN* gene mutations may be a contributing molecular mechanism in human hypertension.

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