A Corin Variant Identified in Hypertensive Patients That Alters Cytoplasmic Tail and Reduces Cell Surface Expression and Activity

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Corin is a membrane-bound protease that regulates blood pressure by activating the natriuretic peptides. **CORIN** variants have been associated with hypertension and heart disease in African Americans. In this study, we conducted targeted exome sequencing and identified an insertion variant, c.102_103insA, in exon 1 of the **CORIN** gene. Analysis of two independent cohorts showed that the variant was preferentially present in hypertensive patients (38/795 or 4.78% vs. 4/632 or 0.63% in normal individuals, \( p < 4.14 \times 10^{-6} \)). The insertion shifted the reading frame, resulting in a corin variant with a truncated cytoplasmic tail. In cell-based studies, the corin variant exhibited poor trafficking in the Golgi, reduced cell surface expression and zymogen activation, and low natriuretic peptide processing activity. Compared with normal individuals with the wild-type allele, individuals with the variant allele had lower levels of plasma corin (0.59 ± 0.07 ng/mL (n = 25) vs. 0.91 ± 0.02 ng/mL (n = 215), \( p < 0.001 \)) and higher levels of plasma N-terminal pro-atrial natriuretic peptide (NT-pro-ANP) (2.39 ± 3.6 nmol/L (n = 21) vs. 0.87 ± 0.6 nmol/L (n = 48), \( p = 0.005 \)).

These results indicate that the variant altered corin structure and impaired the natriuretic peptide processing activity in vivo. The results highlight corin defects as an important underlying mechanism in hypertension.

Hypertension is a major cardiovascular disease, which increases the risk for stroke, myocardial infarction, and heart failure. Genetic factors play an important role in hypertension\(^1\)–\(^4\). To date, molecular defects in most hypertensive patients remain unclear\(^2\),\(^3\), suggesting that more contributing factors are yet to be identified. Atrial and B-type natriuretic peptides (ANP and BNP) are cardiac hormones that regulate sodium homeostasis and blood pressure\(^5\)–\(^6\). In cardiomyocytes, these peptides are synthesized in pro-forms, i.e. pro-ANP and pro-BNP, which are converted to active ANP and BNP by corin, a membrane-bound serine protease\(^7\). The importance of corin in regulating blood pressure has been shown in corin knockout mice, which exhibited reduced sodium excretion and salt-sensitive hypertension due to impaired natriuretic peptide processing\(^8\)–\(^10\).

Structurally, corin belongs to the type II transmembrane serine protease family, a group of trypsin-like enzymes involved in diverse biological processes\(^11\),\(^12\). All these membrane-bound proteases consist of an N-terminal cytoplasmic tail, a single-span transmembrane domain and an extracellular region containing various modules and a C-terminal protease domain. The transmembrane domain anchors the proteases on the cell surface, where proteolytic processes take place\(^11\),\(^12\). It has been shown that cell surface targeting is important for corin zymogen activation and biological activity\(^13\)–\(^15\).

Human **CORIN** gene consists of 22 exons and spans >200 kb in length on chromosome 4\(^5\), making it one of the largest protease genes in the human genome. In principle, natural variants and mutations are more likely to occur in large genes. To date, **CORIN** variants have been associated with hypertension in African Americans\(^16\)–\(^20\). **CORIN** mutations that reduce corin activity also have been found in patient families with hypertension and...
preeclampsia\textsuperscript{21–24}. It remains unknown if additional CORIN variants are present in general populations that may contribute to hypertension.

In this study, we conducted targeted exome sequencing and identified a novel insertion variant in the \textit{CORIN} gene that was preferentially present in patients with hypertension. Biochemical experiments showed that the variant altered corin cytoplasmic tail sequence and reduced corin cell surface expression, zymogen activation, and pro-ANP processing activity. Our results suggest that naturally occurring \textit{CORIN} variants may reduce corin activity and contribute to hypertension in patients.

**Results**

**Identification of \textit{CORIN} Gene Variant.** We sequenced \textit{CORIN} exons and intron-exon boundaries in genomic DNA samples from normal and hypertensive individuals and identified a variant allele with a single adenine insertion at nucleotide position 102 (c.102\_103insA, referred to as “insA variant” hereafter) in exon 1 of the \textit{CORIN} gene (Fig. 1A)\textsuperscript{16,25}. The insertion shifted the reading frame, creating a down-stream stop codon (Fig. 1B).

The variant allele occurred more frequently in hypertensive patients than normal individuals. In the first study cohort, 23 of 401 hypertensive patients (5.74%) were heterozygous for this variant allele, whereas 2 of 217 normal individuals (0.92%) were heterozygous for the variant allele (\textit{p}=0.0037). We verified this finding in a second study cohort. Among 394 hypertensive patients, 15 or 3.81% were heterozygous for the variant allele, whereas 2 of 415 normal individuals (0.48%) were heterozygous for the variant allele (\textit{p}=0.00998). When the data of both cohorts were combined, 38 of 795 hypertensive patients (4.78%) and 4 of 632 normal individuals (0.63%) were heterozygous for the variant allele (\textit{p}=0.00098).

To test this hypothesis, we expressed the variant in HEK293 cells. WT corin (positive) and inactive mutants R801A and S985A (negative) were used as controls (Fig. 1B). Surprisingly, Western analysis detected the insA variant expression in transfected HEK293 cells (Fig. 2A).

**Characterization of insA Variant Corin.** The insertion of an adenine at nucleotide position 102 shifts the reading frame (Fig. 1B), which is expected to prevent corin expression. We made the plasmid, pcDNAinsA, containing the adenine insertion and transfected it in HEK293 cells. WT corin (positive) and inactive mutants R801A and S985A (negative) were used as controls (Fig. 1B). Surprisingly, Western analysis detected the insA variant expression in transfected HEK293 cells (Fig. 2A).

To test if the insA variant corin is biologically active, a pro-ANP processing assay was performed. The variant exhibited pro-ANP processing activity, although the level was markedly reduced compared to that of WT (Fig. 2B). In negative controls (vector, mutants R801A and S985A), little pro-ANP processing activity was observed (Fig. 2B). By densitometric analysis, the variant had 41.3 \pm 8.5% activity compared with that of WT (\textit{p}<0.01) (Fig. 2C).

**Zymogen Activation of Corin Variant.** One possible explanation for the insA variant expression is that a down-stream methionine was used as the translation initiation site. Indeed, there is a methionine at position 30 that may be used as an alternative initiation site (Fig. 1B). To test this hypothesis, we expressed the variant in HEK293 cells together with a corin isoform that contains methionine 30 (Met-30) but lacks methionine 1 (Met-1)\textsuperscript{15}. By Western analysis, similar expression levels of the insA variant, the Met-30 isoform (Met-30), WT, and mutants R801A and S985A were observed (Fig. 3A). On the Western blots, an \textcirca 40-kDa band (corin-p), representing the corin protease domain cleaved at the activation site Arg-801 (Fig. 1B), was detected in WT (Fig. 3A, lane 2). This band also was detected in the catalytic site mutant S985A (Fig. 3A, lane 5), but not in mutant R801A, in which the cleavage site is abolished (Fig. 3A, lane 4). The intensity of this band in the insA variant was markedly

![Figure 1](https://www.nature.com/scientificreports/)
reduced (Fig. 3A, lane 3). Similar results were observed in the Met-30 isoform (Fig. 3A, lane 6). By densitometric analysis, the ∼40-kDa band in WT represented 12.73 ± 1.97% of the total corin protein in lysate, whereas the band in the insA variant was 4.04 ± 1.02%, significantly lower than that in WT (n=5, p<0.01) (Fig. 3B). In the Met-30 isoform, this band was 3.99 ± 2.67% of the total corin protein, which was similar to that in the insA variant (Fig. 3B). The data support that the insertion resulted in a variant corin with a shorter cytoplasmic tail and reduced zymogen activation and activity.

Corin Expression on Cell Surface. Corin is a membrane-bound protease and its activation depends on cell surface expression14. Previous studies indicated that cytoplasmic tail sequences may alter corin cell surface expression15. We examined the variant expression in biotin-labeled HEK293 cells. Surface expression of the insA variant was much less abundant than that of WT (5.5 ± 1.5 vs 31.5 ± 8.4%, n=3, p<0.01) (Fig. 4A and B). Similarly reduced cell surface expression was observed in the Met-30 isoform (6.7 ± 3.1%, n=3, p<0.01 vs. WT) (Fig. 4A and B). As controls, the cell surface expression of mutants R801A and S985A was similar to that of WT (Fig. 4A). In cell lysate, total corin levels were comparable in all samples (Fig. 4A, right panel). As expected, GAPDH was absent in labeled cell surface proteins, but present at similar levels in all lysate samples (Fig. 4A, lower panels).

Figure 2 | Expression and activity of the insA variant in HEK293 cells. (A) Western analysis of corin WT, the insA variant, and mutants R801A and S985A protein levels in transfected HEK293 cells. (B) Pro-ANP processing activity of the insA variant. WT and inactive mutants R801A and S985A were positive and negative controls, respectively. (C) Quantitative data of pro-ANP processing activity were from densitometric analysis. The cropped blots are used in the main figures and full-length blots are included in the supplementary information (Supplementary Fig. S1). Data are representative of at least four independent experiments.

We verified these results by flow cytometry. In HEK293 cells expressing WT, 32.7 ± 8.5% were corin-positive on the surface (Fig. 4C). In contrast, only 7.9 ± 3.7 and 6.9 ± 6.4%, respectively, were corin-positive in cells expressing the insA variant and the Met-30 isoform (n=3, both p values <0.01 vs. WT).

Subcellular Localization of insA Variant. We next examined the intracellular distribution of the variant by immunostaining. In HEK293 cells expressing WT, predominant corin staining was on the cell surface (Fig. 5A). Such staining was absent in vector-transfected cells. In cells expressing the insA variant, corin staining was more prominent intracellularly. By co-staining with TGN46, a Golgi marker, a significant portion of the insA variant was found in the Golgi (Fig. 5A). In parallel experiments of co-staining with PDI, an ER marker, no significant staining of WT or the variant was found in the ER (Fig. 5B). The data indicate that the insA variant was less efficient in trafficking through the Golgi network.

Soluble Corin in Cultured Cell Medium. As reported previously26, cell surface corin undergoes proteolytic shedding, generating three fragments of ∼180-, ∼160-, and ∼100-kDa, respectively, in the conditioned medium. The ∼180-kDa fragment was from a disintegrin and metalloprotease (ADAM)-mediated cleavage, whereas the ∼160- and ∼100-kDa fragments were from corin autocleavage26. We examined corin shedding in HEK293 cells. Three bands of the

Figure 3 | Zymogen activation of the insA variant in HEK293 cells. (A) Western analysis of corin proteins in cell lysate. The ∼40-kDa band represented the corin protease domain (corin-p) from activation cleavage. This band was absent in mutant R801A, in which the cleavage site was abolished. Data are representative of five independent experiments. (B) Percentage of the activated protease domain fragment (corin-p) vs. corin zymogen protein (corin) was estimated by densitometry. Data are mean ± S.D. from five independent experiments.
Figure 4 | Cell surface expression of corin proteins. (A) Western analysis of biotin-labeled corin proteins on the cell surface (left panel) and in cell lysate (right panel). GAPDH was used as a control (lower panels). The cropped blots are used in the main figure and full-length blots are included in the supplementary information (Supplementary Fig. S2). (B) Corin bands on Western blots were analyzed by densitometry. Percentage of corin proteins on the cell surface was estimated. Data are mean ± S.D. from three independent experiments. (C) Analysis of cell surface corin proteins by flow cytometry. Data are representative of three independent experiments.

Figure 5 | Subcellular distribution of corin proteins. (A) HEK293 cells expressing WT and the insA variant were stained for corin (green) and TGN46, a Golgi marker (red). (B) HEK293 cells expressing WT and the insA variant were stained for corin (red) and PDI, an ER marker (green). In both experiments, cell nuclei were stained with DAPI (blue) and vector-transfected cells were used as a negative control. Bar: 5 μm.
predicted sizes were found in the conditioned medium from cells expressing WT (Fig. 6A). In cells expressing the insA variant and the Met-30 isoform, the ~180-kDa fragment was less abundant and the ~100-kDa fragment was hardly visible (Fig. 6A). By densitometric analysis of the Western blots, the percentage of the ~180-kDa fragment (Fig. 6B) or all three soluble corin fragments (Fig. 6C) was significantly lower than that of WT (n=4, p values <0.01). In inactive mutants R801A and S985A, only the ~180-kDa ADAM-cleaved fragment was detected, but not the ~160- and ~100-kDa autocleavage fragments, as predicted. The results indicate that the insA variant had reduced shedding, likely due to impaired cell surface targeting and zymogen activation.

Plasma Soluble Corin and NT-pro-ANP Levels. We also measured plasma corin in individuals with WT and the insA variant CORIN alleles. The levels in 215 normal individuals with the WT allele were 0.91 ± 0.02 ng/mL, whereas the levels in 25 individuals with the variant allele were significantly lower (0.59 ± 0.07 ng/mL, p<0.001) (Fig. 6D). When the 25 individuals with the insA variant allele were separated into normotensive and hypertensive groups, the levels in the normotensive individuals (mean ± range: 0.91 ± 0.04 ng/mL) were higher than that in 23 hypertensive individuals (mean ± SD: 0.57 ± 0.07 ng/mL). However, the sample size in the normotensive group was too small for statistical analysis. In a subset of samples from individuals with the WT allele, plasma corin levels were lower in hypertensive individuals (0.62 ± 0.09 ng/mL, n=60, p<0.01) than that in normotensive individuals (0.86 ± 0.43 ng/mL, n=60).

We next measured plasma levels of NT-pro-ANP in individuals with WT and the insA variant alleles (Fig. 6E). Plasma NT-pro-ANP levels in 48 normal individuals with the WT allele were 0.87 ± 0.6 nmol/L, whereas the levels in 21 individuals with the variant allele were 2.39 ± 3.6 nmol/L, significantly higher than that in the normal control (p=0.005).

Discussion

In this study, we performed targeted exome sequencing and identified an insertion variant, c.102_103insA, in the CORIN gene. The occurrence of this variant allele in the normal controls from two independent cohorts (4/632 or 0.63%) is similar to that in the 1000 genome database (8/1094 or 0.73%, p=0.81). This allele, however, is much more prevalent in hypertensive patients (23/401 or 5.74% in the first cohort and 15/394 or 3.81% in the second cohort, both p values <0.001 vs. normal controls in respective cohorts), suggesting that the variant allele may contribute to hypertension in the patients. Previously, a corin minor allele, T555I/Q568P, is linked to hypertension and heart disease17–19. The T555I/Q568P variant allele appears unique in African Americans, as it is mostly absent in other ethnic groups17. Here we have identified a novel corin variant allele that is associated with hypertension in different ethnic populations.

The insertion of a single adenine at nucleotide position 102 shifts the reading frame, causing a down-stream stop codon. In principle, the insertion may result in a null allele. In functional studies, however, we found that the insA variant was expressed at a similar level to that of WT in HEK293 cells, suggesting that the variant may use Met-30 as an alternative initiation site to produce a corin protein with a

Figure 6 | Shed corin fragments in conditioned medium and plasma levels of soluble corin and NT-pro-ANP. (A) Western analysis of corin fragments in the conditioned medium from cells expressing corin WT, the variant and mutants (upper panel). Corin proteins in cell lysate are shown as a control (lower panel). The cropped blots are used in the main figure and full-length blots are included in the supplementary information (Supplementary Fig. S3). Data are representative of four independent experiments. Western blots were analyzed by densitometry. Percentages of the ~180-kDa corin fragment (B) or all three soluble corin fragments (C) were estimated. Data are mean ± S.D. from four independent experiments. (D) Plasma levels of soluble corin in individuals with WT (Control) and the variant (insA) alleles. (E) Plasma levels of NT-pro-ANP in individuals with WT (Control) and the variant (insA) alleles.
shorter cytoplasmic tail. On Western blots, the variant and WT corin migrated similarly, indicating that Western blots are not sensitive enough to detect such a small size difference in corin cytoplasmic tails. This hypothesis was supported by a similar migration pattern in the Met-30 isoform, which contains Met-30 but lacks Met-1 (Fig. 3A).

Previously, we found that different cytoplasmic tail sequences may alter corin expression and activity on the cell surface55. In our functional studies, the insA variant exhibited low pro-ANP processing activity (Fig. 2), which was likely due to reduced cell surface expression (Fig. 4) and impaired zymogen activation (Fig. 3). By immunostaining, we found that the variant was partially retained in the Golgi, reducing its cell surface targeting (Fig. 5). Since corin zymogen activation occurs on the cell surface but not intracellulary55, these results may explain the reduced zymogen activation and activity of the variant. Earlier, a protein motif, DDNN, which precedes Met-30 in human corin cytoplasmic tail (Fig. 1B), was found to be important for corin cell surface targeting55. Consistent with these data, the insA variant lacking the DDNN motif exhibited reduced cell surface expression and zymogen activation in our experiments.

As a mechanism to regulate corin activity on the cell surface, corin is shed by autophagy and ADAM-mediated proteolysis56. Similar shedding has been reported in other membrane-bound proteases57–59. In this study, we found reduced corin shedding fragments in HEK293 cells expressing the insA variant, consistent with the reduced cell surface expression and zymogen activation of the variant. In agreement with this finding, plasma corin levels were significantly lower in individuals with the variant allele than with WT allele, suggesting that the variant may have poor cell surface expression, impaired zymogen activation, and reduced shedding in vivo.

Low levels of plasma corin antigen and activity have been reported in patients with hypertension and heart disease. Low levels of serum corin also were found to be a predictor for poor outcomes in patients with coronary heart disease. In this study, low plasma corin levels were found in hypertensive patients with the variant allele. Consistently, plasma levels of NT-pro-ANP were much higher in these individuals. These data indicate that the activity of the insA variant is lower in vivo, which may impair natriuretic peptide processing in the individuals with the variant allele.

The ANP signaling pathway is important for salt-water balance and normal blood pressure. Genetic variants and mutations in the genes coding for ANP or its receptor have been found in patients with hypertension and heart disease. Low levels of serum corin also were found to be a predictor for poor outcomes in patients with coronary heart disease. In this study, low plasma corin levels were found in hypertensive patients with the variant allele. Consistently, plasma levels of NT-pro-ANP were much higher in these individuals. These data indicate that the activity of the insA variant is lower in vivo, which may impair natriuretic peptide processing in the individuals with the variant allele.

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