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Original Article

Transcription Factors in the Associative Gene Network of the Renin-angiotensin-aldosterone System in Humans

O. Vishnevsky[1,2], K. Kuzminykh[2], I. Yatsyk[1,3], E. Kondratyuk[1,4] and I. Chadaeva[1*]

[1]*Institute of Cytology and Genetics, Novosibirsk, Russia;* [2]*Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russia;* [3]*Artificial Intelligence Research Center, Novosibirsk State University, Novosibirsk, Russia;* [4]*Research Institute of Clinical and Experimental Lymphology, Novosibirsk, Russia*

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Abstract

Background and objectives: Empirical and theoretical studies can be distinguished among the areas of investigation of the renin-angiotensin-aldosterone system (RAAS) and its relationship with the development of cardiovascular diseases. Theoretical work is based mainly on the bioinformatic analysis of key elements of RAAS (genes, proteins, metabolites), on calculations and predictions of protein interactions, and on mechanisms of RAAS gene expression regulation. An associative gene network based on big data analysis allows us to reveal relationships among the proteins, regulatory pathways, and biological processes acting in RAAS, as well as to identify new diagnostic markers, therapeutic targets, putative molecular mechanisms of the development of RAAS-associated diseases, drug interactions, and drug toxicity.

Methods: The reconstruction and analysis of associative gene networks were performed using ANDSystem. The regulation of RAAS-associated gene expression was analyzed by transcription factor (TF) binding sites (TFBSs) prediction in the proximal promoters of these genes and by studying interactions between TFs themselves using the Ensembl Biomart web service and AnimalTFDB 4.0. The recognition of potential TFBSs in RAAS gene promoters was performed using MoLoTool.

Results: According to the centrality criteria of the RAAS associative gene network, the following proteins were identified as exerting a significant influence on information interplay between network components: IL6, EDN1, TNFA, MK01, LEP, and JUN. Analysis of the ten identified TFs and their TFBSs among the genes in the RAAS network under study revealed clusters of three to 26 genes regulated by them.

Conclusions: Components with the highest values of centrality and vertex degrees were identified in the reconstructed associative gene network of the RAAS, and ten TFs supposed to regulate 26 RAAS genes were determined.

Introduction

The renin-angiotensin-aldosterone system (RAAS) plays a central role in the homeostasis of body fluids.[1–3] The core components of the RAAS include renin, angiotensinogen with its products, angiotensin I and II, angiotensin-converting enzymes ACE and ACE2, angiotensin receptors types 1 and 2, Mas receptor, angiotensins 1-9 and 1-7, and aldosterone. Currently, RAAS is extensively studied.[2,4] One of the priority areas of these studies is the analysis of RAAS at the molecular level in relation to the pathogenesis of arterial hypertension and other cardiovascular diseases.

The bioinformatic analysis of RAAS (genes, RNA, proteins, metabolites) mainly consists of calculations and predictions concerning the interaction of gene expression products with each other, mechanisms of regulating RAAS gene expression, etc. Big data allows us to reconstruct an associative gene network in which we can trace biological processes, proteins associated with each other, and the regulation of components. A classification of gene networks and a detailed overview of associative gene networks are given in the review https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/gene-network. In gen-

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***Correspondence to:** I. Chadaeva, Institute of Cytology and Genetics, Novosibirsk 630090, Russia. ORCID: https://orcid.org/0000-0002-2724-5441. Tel: +7-383-363-49-63 Ext. 1311, E-mail: ichadaeva@bionet.nsc.ru

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eral, it can be said that an associative gene network is a representation of gene relationships based on scientific data in peer-reviewed publications (gene expression or protein interactions). This type of network differs from a gene regulatory network, which focuses on direct regulatory interactions (e.g., transcription factor (TF) binding).[5] Associative networks can reveal patterns of gene behavior and potential functional relationships even if the underlying mechanisms are obscure.[6] The best-known text-mining tools and systems that reconstruct molecular genetic networks are Pathway Commons (https://www.pathwaycommons.org/), STRING (http://string-db.com), Biblio-MetReS,[7] MetaCore (https://clarivate.com/products/metacore/), Ingenuity (https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/), and Coremine (https://www.coremine.com/). For all that, a detailed description of the molecular mechanisms of biological processes, requiring consideration of a wide range of relationships between molecular and genetic objects, is a necessary prerequisite for most research works. Therefore, we used the well-proven ANDSystem package to reconstruct the associative gene network of RAAS.[8,9]

A comprehensive analysis of the RAAS gene network allows us to identify new diagnostic markers and therapeutic targets and shed light on possible molecular mechanisms of RAAS-associated disease development, drug interactions, and drug toxicity. It is important to consider not only the key components of this system, such as renin, angiotensin, aldosterone, and others, but also genes whose proteins regulate the expression of TFs of RAAS genes. These proteins control gene transcription by specifically binding to appropriate TF binding sites (TFBSs). By modulating the transcription levels of a significant number of genes, TFs affect the operation of the corresponding gene networks as a whole. In a number of studies, TF genes were identified among the RAAS genes as active participants in the implementation of hormonal regulation of homeostasis by RAAS. Therefore, the role of TFs in the regulation of RAAS gene expression should be comprehensively investigated.

We used the NCBI Gene database to compile a list of 145 genes associated with RAAS and its components and reconstructed an associative network of genes by applying the freely available ANDSystem software.[8–10] We also analyzed TFs and their binding sites in the genes of the reconstructed associative network of RAAS and identified potential binding sites of 10 TFs in the [−300;1] regions. Furthermore, clusters of genes potentially regulated by these TFs were identified, ranging from three (*ZBTB16*) up to 26 (*PPARG*) genes. Notably, seven out of ten identified TFs had potential TFBSs located in the promoters of genes encoding other TFs, which implies mutual regulation.

Materials and methods

RAAS gene network reconstruction and analysis

As material for the reconstruction of associative gene networks, a list of 145 genes associated with RAAS (Table S1) and its components according to literature data was compiled using the NCBI Gene database.[10] The following queries were used in this study: "RAAS[All Fields] AND alive[prop] AND "Homo sapiens" [porgn:__txid9606]", "renin-angiotensin aldosterone system[All Fields] AND alive[prop] AND "Homo sapiens" [porgn:__txid9606]", and "renin[All Fields] AND alive[prop] AND "Homo sapiens" [porgn:__txid9606]" with the settings "Gene sources": Genomic, "Categories": Annotated genes and Protein-coding, "Sequence content": Ensembl.

We employed the software tool ANDSystem, developed at the Institute of Cytology and Genetics SB RAS, to analyze molecular interactions. The algorithms of ANDSystem are based on data extraction from literature (text mining and data mining, Fig. 1), since biological data reported in scientific literature undergo an independent peer-review procedure.[8,9] The main modules of ANDSystem are the knowledge extraction module, ANDCell knowledge base, and user interface ANDVisio.

We reconstructed an associative gene network for RAAS consisting of 145 genes, which included relations to encoded proteins, as well as TFs regulating the expression of RAAS genes. A more detailed analysis and the identification of the gene regulatory network within RAAS involved additional queries, enabling the recognition of smaller subnetworks. These subnetworks clearly illustrated specific regulatory interactions in closed loops of protein-protein and gene-protein interactions and highlighted mechanisms of self-regulation within this signaling pathway. Such feedback mechanisms stabilized signaling pathway activity, regulated gene expression levels, and enabled adaptive cellular responses to variations in the intracellular and external environment. The gene regulatory networks were also processed with the Pathway Wizard tool included in the ANDSystem software suite. We used the tool with default parameters, which had been optimized by the developers on control data.

Functional annotation of RAAS gene network components

For functional annotation of RAAS-associated genes, we employed the DAVID web service (https://david.ncifcrf.gov/tools.jsp).[11] This tool enables functional annotation and enrichment analysis of gene lists for 55,464 species. With DAVID, we performed a Gene Ontology (GO) biological process enrichment analysis of the gene network, applying Benjamini-Hochberg multiple-comparison correction and setting the significance threshold to 0.05.

We also used the STRING database to functionally annotate the RAAS gene network proteins.[12,13] STRING automatically collects, analyzes, and integrates diverse protein-protein interaction data from scientific literature via text mining, curated databases, experimental sources, and computational predictions. STRING also provides predicted interactions based on protein co-expression data.

Reconstruction of transcriptional regulation networks

For all genes included in the associative RAAS network, promoter nucleotide sequences in the [−2000;1] and [−300;1] regions relative to the transcription start sites were obtained from the human reference genome (GRCh38.p14 assembly) using the Ensembl Biomart web service (https://www.ensembl.org/). Genes encoding TFs were then identified among the RAAS network genes using AnimalTFDB 4.0 (https://guolab.wchscu.cn/AnimalTFDB4/#/).[14] Subsequently, potential TFBSs in the promoters of all genes within the RAAS gene network were predicted using the MoLoTool web service (https://molotool.autosome.org/).[15] The default significance threshold (p-value < 10^{-4}) recommended by the MoLoTool developers, as well as the H13CORE models collection, was applied. Based on the obtained information about potential TFBS locations, we reconstructed transcriptional regulation networks of RAAS genes using the ANDSystem tool.

Results

RAAS gene network reconstruction and analysis

We started from the list of 145 genes reported to be related to RAAS and the list of direct components of RAAS and reconstructed a complete associative gene network of RAAS, which included the 145 genes and the same number of proteins encoded by them. The net-

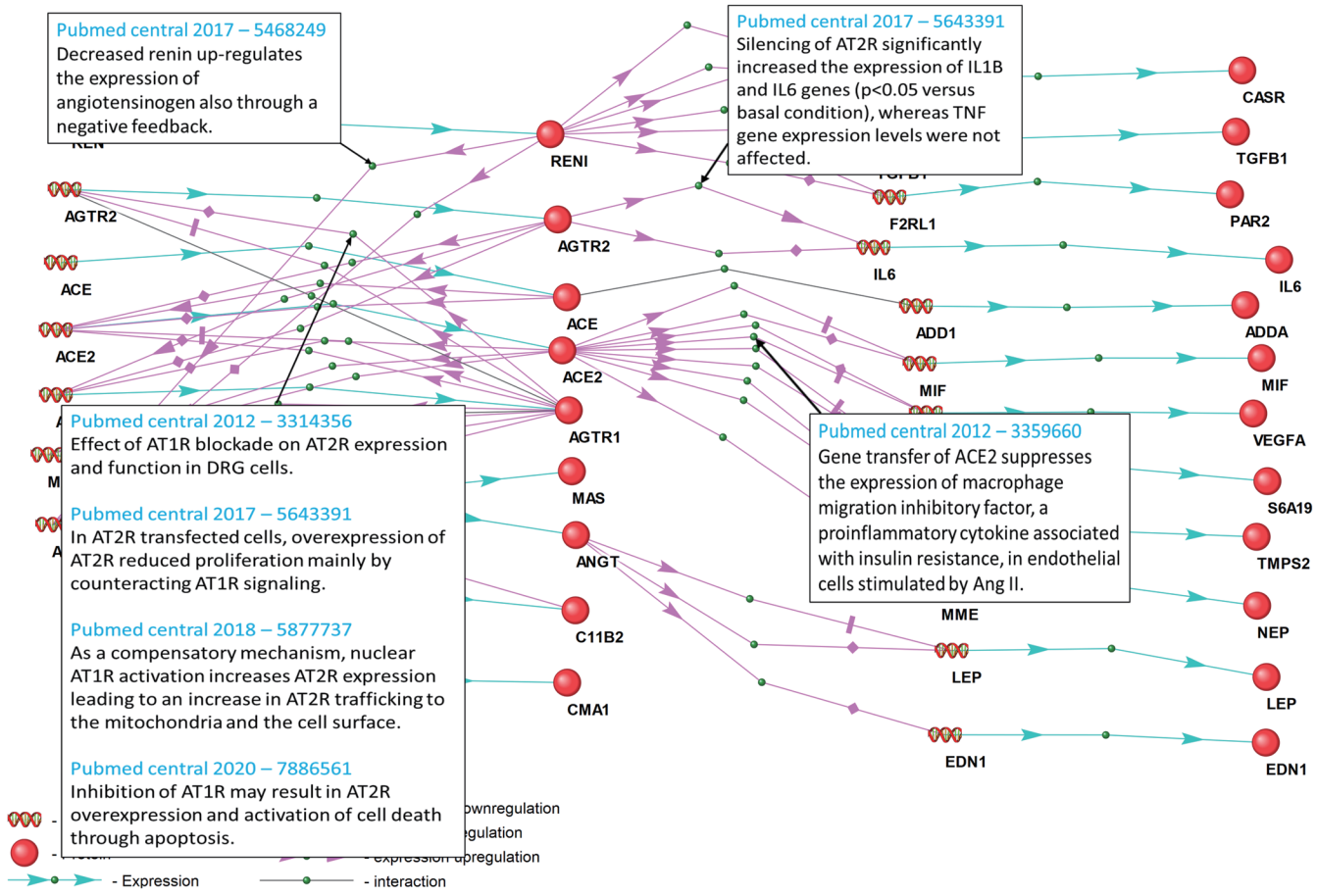

Fig. 1. Gene regulatory network of interaction by the type of expression regulation between the nine core genes of the RAAS and their proteins. Proteins are shown as circles, and genes as DNA helices. Nodes in the network represent genes and proteins. The image was produced with the ANDVisio program, which is part of ANDSystem. The gene/protein notations follow the ANDVisio output. RAAS, renin-angiotensin-aldosterone system.

work was supplemented with 1,457 associations between the genes, such as regulation, catalysis, cleavage, degradation, protein transport, and gene expression, as shown in Figure 2.

One can see in Figure 2 that some genes and their proteins are not associated with other components of the network (e.g., *OCRL*, *ACTN3*); they are placed on the right in separate pairs. First, this may reflect the fact that publications on associations of these genes and/or proteins with other RAAS components were released later than the current version of the public web service ANDSystem we refer to,[8] according to the regulation of its updates by the developers. Second, while the genes under investigation may have been studied in relation to RAAS dysfunction, their association with other RAAS components was not considered. Third, it is possible that the presence or absence of associations between the components of the RAAS gene network has not been the focus of experimental studies so far. The genes of interest may have been studied in the context of association with RAAS dysfunction, but their association with other RAAS components remained unconsidered. Fourth and finally, it is possible that experimental studies have not yet focused on the presence or absence of any associations between the components of the RAAS gene network existing in nature.

In the next step of this study, it would be interesting to use standard research options of the freely available web service ANDSystem in an independent analysis of all components of the associative network for their prioritization according to generally accepted characteristics of vertices in graph theory: the centrality index by mediation and the centrality index by vertex degree. The higher the centrality index by mediation for a certain component, the greater the number of shortest paths between other components of the associative network of the RAAS that pass through this component. Therefore, it may be assumed that components with the highest values of the mediation centrality index are most involved in the transmission of signals between other network components. The second characteristic of components of the RAAS associative network is the vertex degree index. It is used to prioritize the components. It numerically equals the total number of associations of the component in question with all other components of this network. Thus, the component of the RAAS associative network with the highest vertex degree may have the potential to affect the functioning of the largest number of other components of this network. The main result of this step is that the following components of the RAAS associative network with the highest values of both centrality and vertex degree were prioritized: proteins IL6, EDN1, TNFA, MK01, LEP, and JUN. All of them appear to be involved in the processes of inflammation, immune response, vasoconstriction, apoptosis, and cell growth.[16–19]

We also identified genes and proteins in the constructed subnetworks that participate directly in RAAS and conventionally termed

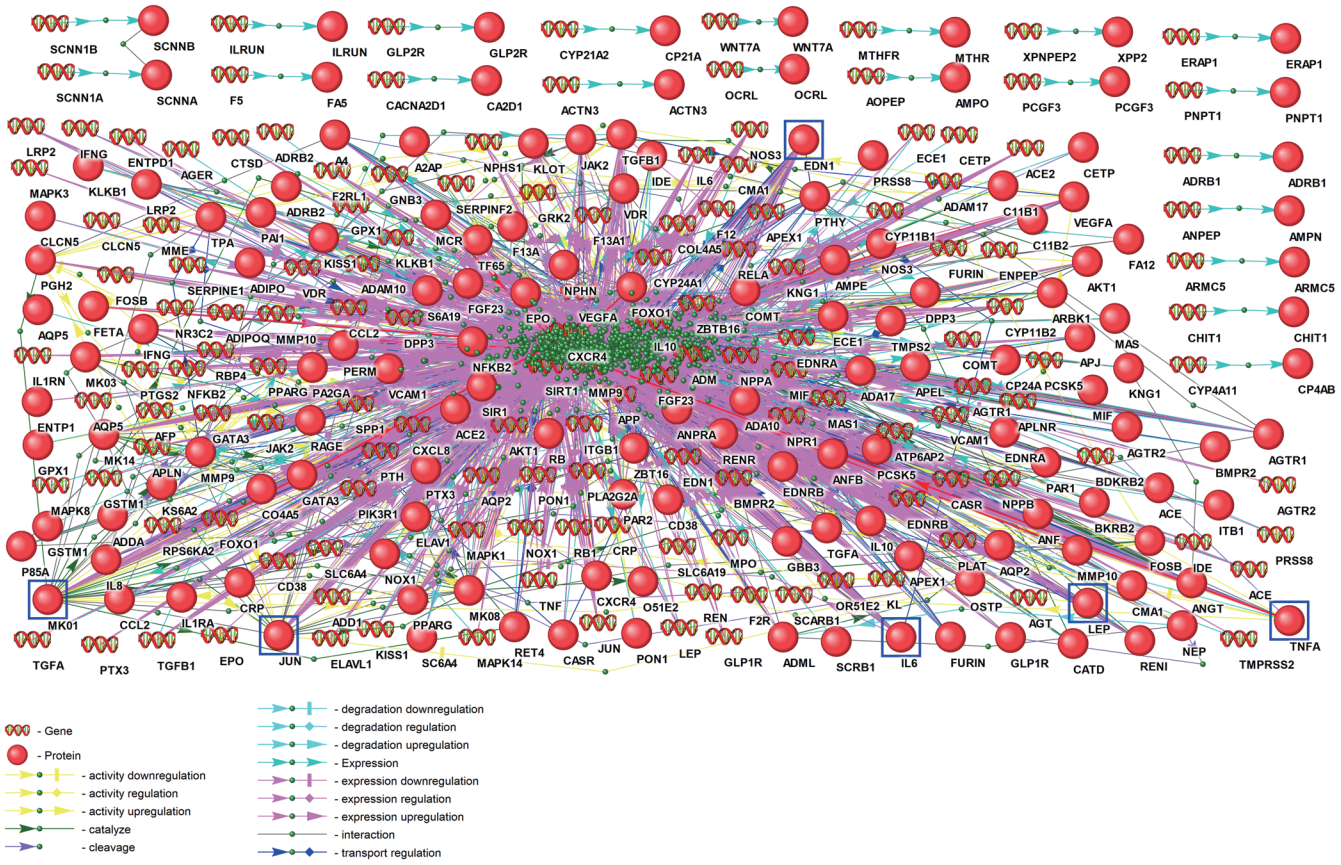

Fig. 2. Associative network of genes (145) of the RAAS, supplemented with interactions between network components (1,457), down- and upregulation of activity, and the regulation of degradation, catalysis, decay, and transport. Analysis of the RAAS gene-protein interaction network reveals key regulators IL6, EDN1, TNFA, MK01, LEP, and JUN. They are shown in blue boxes. All of them are involved in inflammation, immune response, vasoconstriction, apoptosis, and cell growth. Proteins are shown as circles, and genes as DNA helices. The image was generated using the ANDVisio program, part of ANDSystem. The gene/protein notations follow the ANDVisio output. EDN1, endothelin 1; IL6, interleukin 6; JUN, transcription factor JUN; LEP, leptin; MK01, mitogen-activated protein kinase; RAAS, renin-angiotensin-aldosterone system; TNFA, tumor necrosis factor alpha.

them the "*core*". They include the genes *ACE*, *ACE2*, *AGTR1*, *AGTR2*, *AGT*, *MAS1*, *REN*, *CYP11B2*, and *CMA1* (nine in total); their proteins ACE, ACE2, AGTR1, AGTR2, AGT, MAS, RENI, C11B2, and CMA1; and proteins formed as a result of enzymatic transformations: Ang I, Ang II, Angiotensin 1-7, and Angiotensin 1-9. To study the sequence of interactions between these key components of the RAAS, we built a gene regulatory network. In the interface of the ANDSystem tool, the path length was set equal to the number of objects, 9. Additionally, the classes of objects and types of interactions between them were set. Thus, we obtained the following pipeline: {core genes of the RAAS} → {expression} → {human proteins} → {interactions, expression, and its regulation} → {human genes} → {expression} → {proteins of the associative network of the RAAS}, to simplify and systematize the complete associative network of RAAS (Fig. 2) for *in silico* expression analysis, as depicted in Figure 3.

The RAAS gene regulatory network includes interactions between genes involved in the up- and downregulation of expression. This network contains 21 genes, the same number of proteins, and 63 interactions. The gene regulatory network of interaction between gene expression regulation and protein components of the RAAS associative network repeats the previously constructed RAAS gene network (Fig. 2), allowing a more detailed examination of the interactions of expression regulation of the genes of our interest with their partners in biochemical reactions in the cell. The RENI and ACE2 proteins are the most central proteins of the network, as they affect the expression of several genes of the RAAS network, whereas the AGTR1 protein significantly affects the expression of the nine aforementioned core RAAS genes, but not other genes of the network. No TFs were found in the final segment of the regulatory network. Since no direct link between the core RAAS genes and TFs was found in the resulting network (Fig. 3), additional steps were included in this regulatory network to take into account intermediaries between the core RAAS genes and other RAAS-associated genes (Fig. 4): nine core RAAS genes → expression → human proteins → gene expression regulation, protein activity, catalysis, degradation and transport, interactions → human proteins → expression and its regulation → RAAS-associated genes.

It is seen in Figure 4 that the transcription-related extension of the primary gene regulatory network contained 83 genes, 58 proteins, 611 interactions, one metabolite, and two metabolic processes. According to current biological publications in factual databases, an information extract of which is the ANDSystem, we can see how the core RAAS genes (the rightmost column) can regulate, via intermediary proteins, virtually all TFs involved in RAAS with the only exception being the human *FOSB* gene. The regulatory

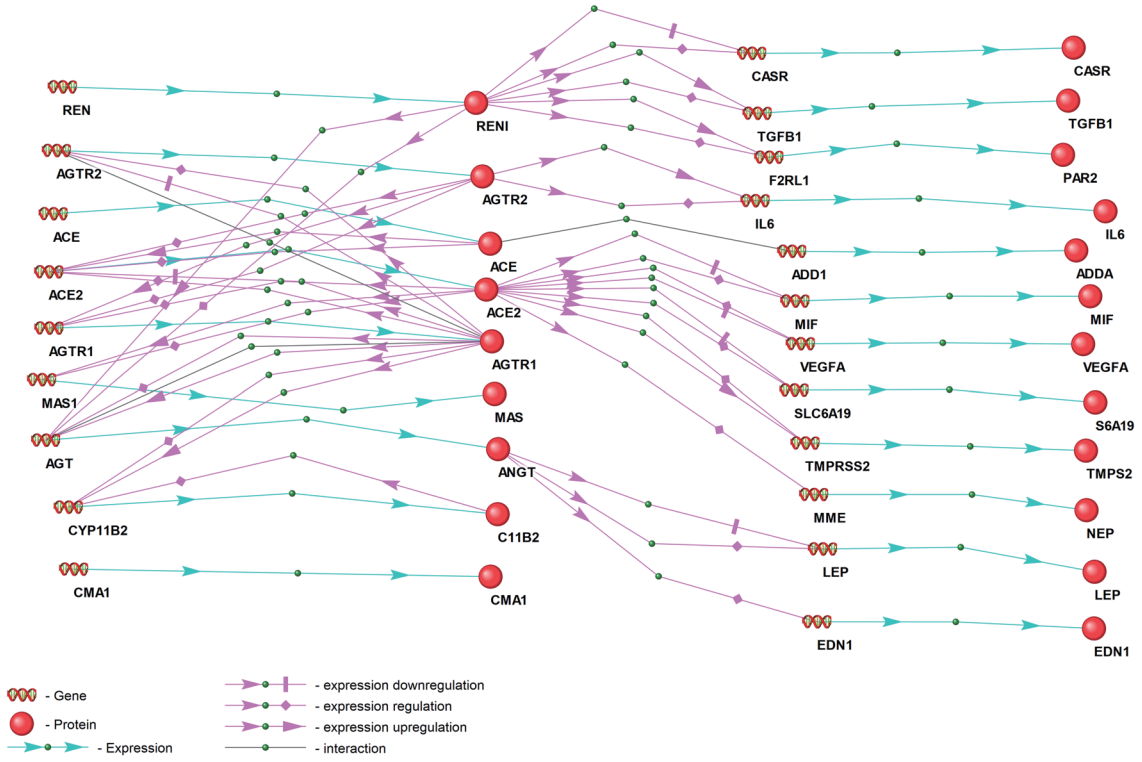

Fig. 3. Gene regulatory network of interaction by the type of expression regulation between the nine core genes of the RAAS and their proteins. Proteins are shown as circles, and genes as DNA helices. Nodes in the network represent genes and proteins. The image was generated with the ANDVisio program. The gene/protein notations follow the ANDVisio output. RAAS, renin-angiotensin-aldosterone system.

mediator proteins from the core of the RAAS associative network to the *FOXO1* TF gene appeared to be IGF1 and P53. For the *VDR* gene, the mediators were TGFB1; for *ZBTB16*, P53; for *GATA3*, IL15, IL33, and SCF; for *RELA*, TGFB1; for *PPARG*, Substance P, Ang II, and matrix metallopeptidase 9 (MMP9); for *NR3C2*, ANGII. For the *JUN* gene, the mediator proteins were TF65, MYC, and OCLN; for the *NFKB2* gene, MYC.

Thus, due to the transcription-related part of the gene regulatory network of RAAS constructed in this work (Fig. 4), we showed that the core of the associative gene network of RAAS could indirectly regulate TFs involved in the entire RAAS gene network (Fig. 2). This finding fits a wide range of independent experimental data on particular cases of such regulation.

It is pertinent to note that the gene for TF *FOSB* was outside the transcription-related part of our gene regulatory network of RAAS (Fig. 4). However, the authors of a biomedical human disease model using rats described a downregulation of this gene simultaneously with a losartan-induced block of angiotensin type 1 receptor AGTR1,[20] which is part of the core RAAS genes listed in the leftmost column in Figure 4. Thus, we assume that the extension of the complete associative gene network of RAAS (Fig. 2), which presently encompasses only natural substances, to synthetic medications, food supplements, and environmental pollutants, may be a promising next step in further inquiry in this field.

Methodological limitations of the ANDSystem program: The ANDSystem relied on specific formulations of particular interactions between components of the associative gene network. ANDSystem extracted these formulations from publications. As an example of ANDSystem's methodological capabilities, Figure 1 shows specific phrases from articles in text fields describing the regulation of *AGTR2* gene expression by the AGTR1 protein, the upregulation of *AGT* expression by renin, the regulation of *MF* gene expression by ACE2, and the regulation of the *IL6* gene by AGTR2.

Functional annotation of RAAS gene network components

The next step of this study was an analysis of the overrepresentation of GO terms of the "biological process" type. We performed it using the widely accepted web tool DAVID.[11] As a result, the set of 145 RAAS-related human genes in question was most significantly enriched in the following top five GO terms:

- GO:0008217 ~ regulation of blood pressure (*p*-value = 7.6E-26);
- GO:0006954 ~ inflammatory response (*p*-value = 7.5E-15);
- GO:0042311 ~ vasodilation (*p*-value = 3.3E-14);
- GO:0006508 ~ proteolysis (*p*-value = 1.1E-12);
- GO:0001666 ~ response to hypoxia (*p*-value = 1.8E-14).

The first four of the five most reliable GO terms are generally recognized biological functions of RAAS. The association of RAAS with the fifth term can be attributed to the active renal production of renin under hypoxic conditions, which triggers the RAAS cascade.[21]

Thus, the identified GO terms accurately described the key role of RAAS in the regulation of blood pressure, inflammatory responses, adaptation to hypoxia, and proteolytic processes.

Reconstruction of transcriptional regulation networks

The analysis of the list of genes of the RAAS associative network revealed 10 genes encoding human TFs (Table 1).

We recognized potential TFBSs for each of the nine TFs listed

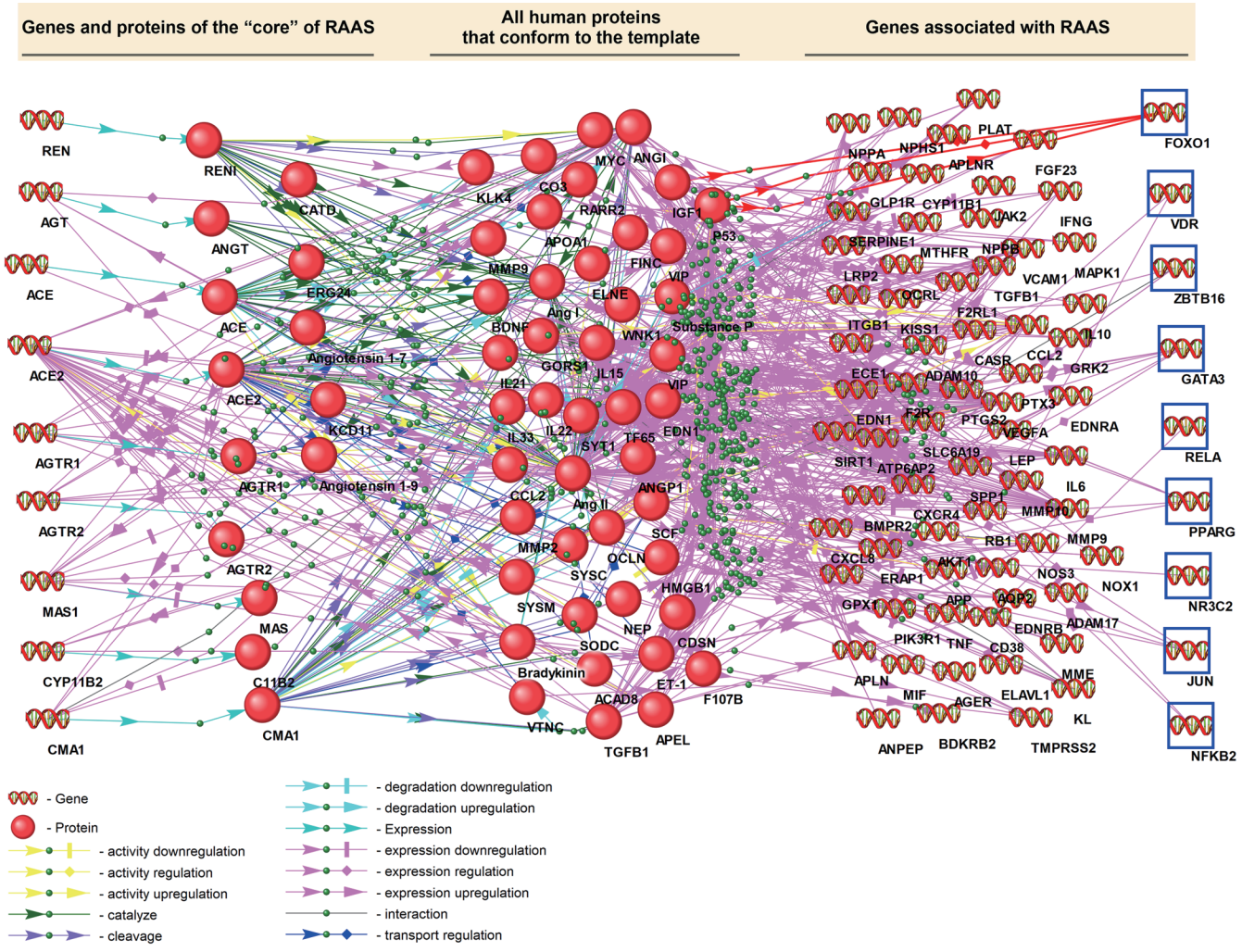

Fig. 4. Gene regulatory network of intermediaries between the core RAAS genes and other RAAS-associated genes. The rightmost column of the gene regulatory network contains the only genes for TFs, shown in blue boxes. Proteins are shown as circles, and genes as DNA helices. Nodes in the network represent genes and proteins. The image was generated using the ANDVisio program. The gene/protein notations follow the ANDVisio output. RAAS, renin-angiotensin-aldosterone system; TFs, transcription factors.

within the rightmost column in Figure 4, along with one more TF, FOSB, taken additionally into account on the grounds of biomedical data from rat models of human diseases,[20] as it interacts with AGTR1. For all 10 TFs, potential TFBSs were predicted in the promoters of genes included in the RAAS associative network, gene clusters conceivably regulated by these TFs were identified (Table 1), and transcriptional regulatory networks were reconstructed (Fig. 5).

According to the data presented in Figure 5 and Table 1 (column N_{300}), the numbers of genes potentially regulated by RAAS TFs varied from three for *ZBTB16* to 26 for *PPARG*. Seven out of the ten considered TFs could also regulate other RAAS-related TFs. For example, the JUN TF could potentially regulate three other RAAS-related TFs, which was the highest estimate in this study.

Interestingly, JUN and RELA TFs could conceivably self-regulate. JUN and FOSB TFs could potentially regulate each other, forming a closed loop of mutual regulation. The "TF_RAAS_Gene2000" column of Table 1 shows a significant increase in the number of RAAS-related genes in whose indicated proximal promoter regions potential TFBS were detected, namely from 42 RAAS genes (for *ZBTB16*) to 108 (for *PPARG*), among a total of 145. In addition, the number of RAAS-related TFs that could potentially be targets for regulation by other RAAS-related TFs in our framework ranged from one for ZBTB16 to nine for PPARG, as well as for NFKB2. Similarly, among the 10 TFs studied here, the number of those potentially capable of autoregulation increased to six. Thus, the simplified RAAS-related transcriptional regulatory network, considering only 300-bp as the proximal promoter region, more or less adequately reflected the main features of the entire regulatory network as a whole. All other regulatory regions predominantly modulated only individual details of RAAS functioning, in accordance with modern concepts of eukaryotic transcription regulation. Finally, it was found that the single JUN TF was a potential target for all such TFs except for the ZBTB16 TF.

Discussion

Studies of RAAS where gene networks are employed are few. Some scientists explored only individual elements of RAAS us-

Table 1. Transcription factors and examples of their potential target genes in the RAAS associative network

TF symbol	N_{300}	$TF\_RAAS\_Genes_{300}$	N_{2000}	$TF\_RAAS\_Genes_{2000}$
NR3C2	5		56	*GATA3, JUN, PPARG, VDR, ZBTB16*
NFKB2	12	*PPARG, RELA*	68	*FOSB, FOXO1, JUN, NFKB2, NR3C2, PPARG, RELA, VDR, ZBTB16*
ZBTB16	3		42	*NR3C2*
PPARG	26	*VDR*	108	*FOSB, FOXO1, GATA3, JUN, NFKB2, NR3C2, PPARG, VDR, ZBTB16*
RELA	16	*NR3C2, RELA*	76	*FOSB, FOXO1, JUN, NFKB2, NR3C2, RELA, VDR, ZBTB16*
VDR	25	*FOSB*	103	*FOSB, GATA3, JUN, NFKB2, NR3C2, PPARG, RELA, ZBTB16*
FOXO1	9		69	*FOXO1, JUN, RELA, ZBTB16*
JUN	13	*FOSB, JUN*	63	*FOSB, GATA3, JUN, NFKB2, RELA, VDR, ZBTB16*
FOSB	17	*JUN*	64	*FOSB, GATA3 JUN, NFKB2, PPARG, VDR, ZBTB16*
GATA3	10	*JUN*	51	*JUN, PPARG, VDR*

N_{300} – number of RAAS genes in whose [–300;1] promoter regions a potential TFBS was found; $TF\_RAAS\_Genes_{300}$ – transcription factor genes in whose [–300;1] promoter regions a potential TFBS was found; N_{2000} – number of RAAS genes in whose [–2000;1] promoter regions a potential TFBS was found; $TF\_RAAS\_Genes_{2000}$ – RAAS genes in whose [–2000;1] promoter regions a potential TFBS was found. RAAS, renin-angiotensin-aldosterone system; TF, transcription factor; TFBS, transcription factor binding site.

ing the reconstruction of an associative gene network. Examples include works dedicated to the function of ACE2 in mice,[22] evaluation of the expression of genes associated with AGTR1 in mouse kidneys, juxtaglomerular cells secreting renin using immunohistochemistry, and potential targets of RAAS inhibitors in the context of diabetic nephropathy treatment.[23,24] Another study using a gene

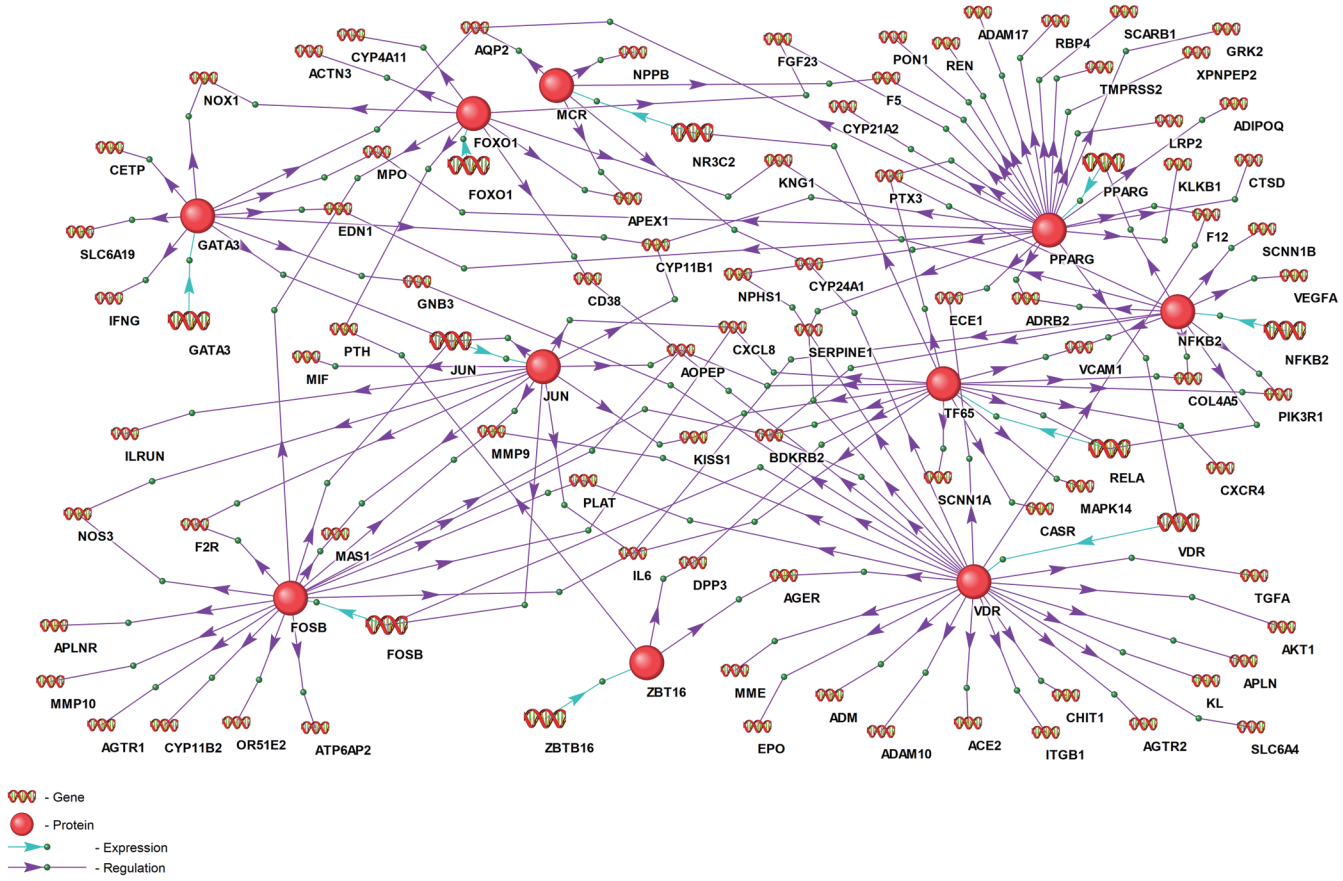

Fig. 5. Gene regulatory network of intermediaries between the transcription factor genes based on predictions of potential TFBSs in proximal promoters of RAAS-associated genes. Proteins are shown as circles, and genes as DNA helices. Nodes in the network represent genes and proteins. The image was produced using the ANDVisio program. The gene/protein notations follow the ANDVisio output. RAAS, renin-angiotensin-aldosterone system; TFBS, transcription factor binding site.

network including RAAS components brought us closer to understanding the genetics of diabetic nephropathy development.[25] In still another study, analysis of gene networks associated with the development of essential hypertension revealed a close involvement of a gene network centered on the NOS3 protein.[26] Here, we focus on reconstructing an associative network of 145 genes included in RAAS in order to evaluate interactions between the components of this system based on the analysis of a large body of data. Gene networks are groups of coordinated genes that interact with each other via their primary products (RNA and proteins), various metabolites, and other secondary products of gene network functioning. As such, they can be helpful in obtaining information about the mechanisms of biological processes and, as a consequence, the development of diseases, identification of new diagnostic markers of diseases and their targets, detection of side effects of drugs and the comorbidity of diseases, and interpretation of omics data.[27] Thus, the gene network provides many opportunities for a comprehensive study of biomedical issues.

Here, we reconstructed a complete associative network of genes associated with RAAS (Fig. 2) within the scope of the freely available web service ANDSystem,[8] used here to reflect the current state of scientific publications and databases on biology involving a data mining approach. By analyzing Figure 2, we ensured that the ultimately reconstructed associative gene network as a whole is consistent with the concept of a feedback mechanism in RAAS: an increase in the rates of reactions caused by increased expression of genes encoding proteins of the classical pathway activates the counter-regulatory pathway, and a decrease in the expression contributes to a decrease in the effects of the latter.[1]

First, we prioritized eight components of the RAAS-related network with the highest estimates of both centrality and vertex degree: TNFA, EDN1, MK01, JUN, LEP, IL6, Ang II, and MMP9 proteins. The identified key components might exert the greatest effect on the functioning of the system, interacting with a large number of other network components. As centrality in the associative network is based on text mining in freely available published data, we conclude that it is the functioning of these eight proteins within RAAS that has been most studied to date. For example, the connection between RAAS and cytokines was best demonstrated during the coronavirus pandemic. The binding of SARS-CoV-2 to ACE2 receptors present throughout the body triggers an inflammatory response. This interaction suppresses ACE2 and the anti-inflammatory arm of RAAS but enhances the pro-inflammatory effects of Ang II (Ang II is known to generate oxidative radicals via AGTR1 and is involved in inflammatory processes). This leads to further production of inflammatory cytokines and promotes a cytokine storm through the activation of alveolar macrophages and secretion of three main cytokines: IL-1, IL-6, and tumor necrosis factor alpha. The enormous production of cytokines causes cytokine release syndrome, or *cytokine storm*.[16] EDN1, an endogenous polypeptide, acts as a vasoconstrictor and exhibits a mitogenic effect. It exerts positive inotropic and chronotropic effects on the heart and stimulates the sympathetic and RAAS, being an important regulator of homeostasis.[28] Accumulated data point to a significant role of *MMP* family genes as Ang II targets: cold exposure increases RAAS activity, thereby enhancing Ang II expression, but MMP9 is also upregulated in response to cold. MMP9/TIMP1 imbalance plays a key role in initiating cold stroke in hypertension.[17] Another target is LEP, a hormone produced primarily by adipocytes and involved in a wide range of physiological functions, especially in the regulation of energy balance. LEP stimulates aldosterone production in obesity, thereby creating a positive feedback loop for obesity-associated cardiovascular disease. LEP appears to be a direct regulator of adrenal aldosterone production, and LEP-mediated aldosterone production is a potential mechanism underlying obesity-associated hypertension, particularly in women.[29]

Our associative gene network of RAAS (Fig. 2) also contains some network components, each of which can participate only in particular interactions, e.g., the *VEGFA* gene in the category "Enhanced gene expression", FOXO1 proteins in the category "Decreased protein activity", and ELAV1 in "Regulation of protein degradation". Apparently, they play specific roles in certain pathways or reactions. It seems reasonable to assume an important modulating role for such specialized components, for example, to ensure mutual consistency of the joint functioning of RAAS simultaneously with the work of other systems within the human body as a whole (e.g., angiogenesis).

According to the annotation in GO terms, the most important processes of the RAAS gene network can be highlighted: blood pressure regulation, inflammatory response, vasodilation, proteolysis, and response to hypoxia. Thus, GO annotation helps us display RAAS in the full range of functions performed by this system.

As for the transcriptional regulation of RAAS genes, in this work we pioneered the reconstruction of the pathways by which RAAS-related core genes can be indirectly regulated by TFs (Fig. 4). We complemented them for the first time with pathways by which these TFs can regulate the expression of all 145 RAAS-related genes (Fig. 5 and Table 1). This is the main result of our study. To this end, we analyzed in more detail how RAAS-related TFs can find their potential binding sites within 300-bp proximal promoter regions upstream of the major transcription start sites in the 145 RAAS-related genes under consideration. Many studies of RAAS TFs have focused on their association with disease development. In particular, Wu *et al.*[30] predicted potential TFs of the *NPPB* gene (precursor of natriuretic peptide B) in humans containing a fragment of the rs3753581 (-1299G) region. According to their results, there were eight such factors, of which three — TFs IRF1, PRDM1, and ZNF263 — are at least partially involved in the development of cardiovascular diseases. However, our work was primarily dedicated to the analysis of the pathways by which TFs in the RAAS can regulate the expression of genes involved in the renin-angiotensin-aldosterone regulation of homeostasis.

To assess how our predictions of the presence of potential TFBSs agree with currently known TFBS localization data obtained from ChIP-Seq experiments, we used the publicly available GTRD gene transcription regulation database (http://gtrd.biouml.org:8888/).[31] This resource contains a rich collection of uniformly processed ChIP-seq data on the identification of TFBSs for humans, mice, and other organisms. Its convenient web interface with advanced search, browsing, and genome browser based on the BioUML platform allows efficient analysis of gene regulatory sequences. Unfortunately, GTRD does not provide the ability to locate TFBSs within arbitrary boundaries, so we confined ourselves to its data on the location of experimentally confirmed TFBSs within [−500;+50] relative to the start of human gene transcription. The results are shown in Table S2.

Table S2 shows that not all potential TFBSs have received experimental confirmation. The proportion of RAAS network genes whose proximal promoters contained experimentally confirmed TFBSs varied from 0 for NR3C2 and ZBTB16, which had the smallest number of predicted TFBSs, to 0.94 for RELA. In general, for the TFs considered, the proportion of confirmed TFBSs was 0.42. Note that the proportion of TFs whose binding sites were experimentally confirmed in the proximal promoters of TFs of the RAAS network was 0.6. We believe that this result is due to several

causes. Firstly, it can be explained by the incompleteness of existing experimental databases, which cannot contain information on the *in vivo* location of TFBSs in all tissues, conditions, and developmental stages of the body. Secondly, such potential TFBSs may represent a certain stored variability of the regulatory systems of a biological species, where these potential TFBSs can start working when the surrounding nucleotide context changes, quickly forming new metabolic regulatory circuits.

In addition, our search for potential TFBSs in proximal promoters did not take into account tissue specificity of gene expression, chromatin accessibility, or other features of the functioning of regulatory regions of RAAS genes. In our study, we identify potential regulatory links that require experimental confirmation.

It should be noted that the search for TFBSs in the [−2000;1] region of gene promoters allows us to compile a deliberately large list of TFs that are involved in one way or another in regulating the expression of target genes. However, upon closer examination, it is not always possible to assess properly the entire contour of the TF–gene interaction. Therefore, we analyzed the TFBSs in the [−300;1] region of gene promoters in more detail in order to obtain a clearer picture of the regulation of RAAS genes.

The regulation of RAAS gene expression by TFs has not been sufficiently studied thus far. There are reports devoted to several TF genes, such as *SOX6* and *ERRα*, whose action is confined to rather narrow ranges. For example, it has been shown that SOX6 TF modulates renin gene expression in renal tissue: SOX6 has a binding site in the REN promoter in the super-enhancer region.[32] The ERRα TF is an important regulator of two vital components of renal blood pressure control: Na^+/K^+ ion homeostasis and the renin-angiotensin pathway.[33] However, no systemic analysis of the interactions of all TFs with RAAS genes has yet been carried out. Therefore, our results may be of pioneering importance in this area and set the tone for further research.

Conclusions

We reconstructed the associative network of the RAAS genes based on information from publications and databases by using the ANDSystem computer program developed at the Institute of Cytology and Genetics, Novosibirsk. We found that the IL6, EDN1, TNFA, MK01, LEP, and JUN proteins have the highest mediation values, which point to their significance in transmitting information between other network components. We also analyzed TFBSs in the proximal [−300;1] promoters of the RAAS genes and interactions between the TFs themselves in order to understand mechanisms regulating the expression of genes associated with RAAS. Bioinformatics analysis of TFs and their binding sites among the genes in the resulting associative network of RAAS revealed 10 TFs that appear to regulate clusters of genes. This result provides new information on RAAS mechanisms with regard to the coordinated and finely regulated interaction of RAAS components themselves, i.e., genes, their proteins, and their regulators, TFs.

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Conflict of interest

The authors declare no conflict of interest. The authors confirm that the submitted manuscript does not violate any copyright agreements.

Author contributions

Conceptualization (IC, OV), data curation and formal analysis (KK, OV), investigation (IC, OV, IY, KK), methodology (IC, KK, IY, OV), supervision (IC), manuscript draft (IC, OV, KK, EK), manuscript review and editing (IC, OV, KK, EK). All authors have read and agreed to the published version of the manuscript. All authors agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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