Journal of Wasit for Science and Medicine 2024: 17, (3), 1-16

Identification of Candidate Transcription Factors that Bind to the ASCN Gene, Associated with Parkinson's Disease, Using Bioinformatics Analysis

Muna A. Abdal Rhida

Wasit University, College of Science, Department of Biology.

Abstract

Parkinson's disease (PD) is a neurodegenerative illness marked by progressive damage of dopaminergic neurons in the substantia nigra. Synuclein-α protein plays a key role in this term by aggregating in clumps of Lewy bodies causing PD. Despite unclear etiology of PD, growing indications show that PD pathogenesis is associated with gene expression dysregulation. Transcription factors (TFs) are the key players in regulating gene expression. In this study, we employed a bioinformatics tool to predict TF binding to Synuclein-α (SNCA)gene utilizing DNA sequences, epigenetic modifications, TF binding motifs, and creating machine learning algorithms. PROMO database was utilized to identify candidate TFs. Here we found TFs that act as regulators of neuronal function and dopaminergic signaling pathways, including members of the Forkhead box family, and nuclear factor-kappa B family members such as c-Jun, and STATs family. These findings provide a better understanding of the molecular mechanisms underlying PD disease and determine potential therapeutic targets.

Keywords: ASCN, Parkinson’s Disease, Bioinformatics, PROMO, Transcription Factors.

1. Introduction

PD is a degenerative neurological disorder that largely impacts an individual's ability to move [1]. This disease affects millions of people globally. It is characterized by the progressive deterioration of neurons that produce dopamine in the substantia nigra part of the brain. The symptoms of the decrease in neurons dopamine are represented by rigidity, bradykinesia,

tremors, and problems with balance and synchronization due to its role in regulatory movement and coordination. Not only motor symptoms, but also non-motor symptoms might appear on the patients such as cognitive impairment, sleep problems, changes in mood, and autonomic dysfunction like fluctuations in blood pressure and gastrointestinal issues [2]. The exact reason behind PD is still ambiguous, however, it is thought to result from a combination of genetic and environmental stimuli. Some genetic mutations are linked with familial variations of the disease. However, most cases arise randomly. Some environmental factors such as exposure to toxins or pesticides might contribute to the heightened risk of emerging PD [3]. On the other hand, the neurons that produce dopamine, an important neurotransmitter for controlling movement, gradually break down in PD. The specific reason of this decline is not identified, but the presence of Lewy bodies comprising misfolded (SNCA) is a vital factor [4]. These clusters of protein restrict the regular cell processes, causing glitches in nerve cells and, eventually, the decease of the cell [5,6].The important role of (SNCA) in PD pathology has made it a promising target for potential therapy [7]. Yet, generating effective therapies presents major obstacles. Efforts to reduce (SNCA) clumping or expand its removal have been fruitful in animal studies but have not confirmed to be operative in human trials. It is also still a challenging work to target (SNCA) without interfering with its biological function. Despite these complications, researchers are still positive about the promises of advanced therapeutic strategies. Progressions in gene therapy, antibody-based treatments, and small molecule inhibitors offer new approaches for addressing (SNCA) pathology [8]. Moreover, current medicines and examining complex treatment options could enhance benefits in addressing the progress of PD [9]. Recent advancements in genomics and computational biology have encouraged investigations into the complicated molecular mechanisms underlying PD pathogenesis, with a precise focus on transcriptional regulation [10]. The dysregulation of gene expression has arisen as a dominant feature in the complex scenery of PD pathology. TFs, as master regulators of gene expression, employ accurate control over the transcriptional machinery, arranging the elaborate networks of gene regulation that support cellular function [11]. Considerate the interplay between TFs and genes implicated in PD holds noteworthy promise for unraveling the molecular details of the

disease and identifying de novo therapeutic targets. For instance, a recent study suggests that the activation of NF1 transcription by FOXA1 leads to the inhibition of the MAPK signaling pathway which eventually ameliorate neuronal damage and motor disability in PD. These results could present innovative concepts in the area of PD administration [12].

Predicting TFs binding to PD-associated genes signifies a cutting-edge tactic to decipher the regulatory architecture of PD. By utilizing genomic data, such as DNA sequences, epigenetic modifications, and TF binding motifs, predictive models can infer putative TF-gene interactions specific to PD, providing respected insights into the transcriptional dysregulation that is fundamental for disease progression. This study sets the stage for discovering the predictive TFs binding to (SNCA) gene implicated in PD, using PROMO database, highlighting its potential to reveal new regulatory mechanisms and therapeutic approaches involved in this overwhelming neurological disorder.

2. Methodology

2.1 The using of PROMO version 3.0 to predict TFs binding to (SNCA) gene.

Comprehending and modeling the gene expression regulatory networks in cells and tissues is one of the most difficult parts of genome biology. Thus, identifying transcription factor binding sites in DNA sequences precisely is a critical process. In order to anticipate binding sites in sequences, researcher can utilize existing knowledge of target sequences in gene regulatory regions to several databases store sets of known binding sites. For instance, TRANSFAC which has the most inclusive collection of DNA binding sites in eukaryotes. The active way to represent binding sites is through positional weight matrices, as they capture the frequency of nucleotides in recognition sites, considering the inherent variability in protein recognition signals. Weight matrices are designed in PROMO by using binding sites from TRANSFAC to find potential binding sites in sequences [13]. There are multiple tools available for predicting transcription factor binding locations with weight matrices. However, PROMO stands out due to its exceptional characteristics. PROMO has many options, such as choosing sites from any species or group of interest, generating matrices automatically for the selected taxonomic level, giving details on other genes regulated likewise, and comparing multiple sequences at once [14].

The initial step in RPOMO database involves selecting the species or taxonomic level for both factors and binding sites. This step is made possible with the aid of a Java applet that can be available from the main menu under ‘SelectSpecies’. In this study, human species was selected and then the matrices were created dynamically which was examined afterword by utilizing the ‘ViewMatrices’ function as shown in figure 3, A-D. The Homo sapiens (SNCA) RefSeqGene sequence was retrieved from NCBI (Reference Sequence: NG_011851.1), then, this sequence was typed in the ‘SearchSites’ form page as shown in figure 2. The query sequence was examined for regions that closely resemble the matrices [15]. In order to speed up the search, automata were used to cover every potential subsequences from the query sequence that meet or exceed the similarity threshold to any of the matrices figure 3. The outcome included a graphical depiction of the anticipated binding sites, projected values for evaluating the importance of the matches, and genes list that were recognized to be controlled by the TFs identified in the predictions. These genes were shown separately or in combination. Data from other genes are extremely advantageous, as detecting useful connections between these genes and the gene in focus could designate important findings. PROMO main menu has an additional option to view the uniqueness of matrices obtained from several organism types, known as 'MatrixSpecificity', as well as a help page [16].

3. Results

Exploitation an inclusive dataset containing genomic sequences, epigenetic marks, and TF binding motifs, we created predictive models to elucidate TF-gene interactions related to PD pathogenesis. The Homo sapiens (SNCA) RefSeqGene sequence was retrieved from NCBI (Reference Sequence: NG_011851.1). This sequence was submitted to PROMO Database to identify putative TF biding sites on SNCA gene sequence. We were able to identify candidate TFs based on the incidence of enriched TF binding motifs. These TFs include GR-α, TFIID, SRY, TCF-4E, GR, AP-2α-A, NFI/CTF, C/EBPβ, ENKTF-1, FOXP3, PR B, PR A, c-Ets-2, IRF-1, NF-AT1, ATF3, IRF-2, GR-β, NF-1, Pax-5, p53, C/EBPα, LEF-1, c-Jun, GATA-1, RXR-α-synuclein, c-Myb, STAT5A, HNF-3α-, TFII-I STAT4, c-Ets-1, Elk-1, PU.1, T3R-beta1, YY1, NF-AT2, STAT1β, HNF-4α, and TCF-4. Some of these TFs are regulators of neuronal functions and many are

dopaminergic signaling pathways regulators
as demonstrated in figure1.

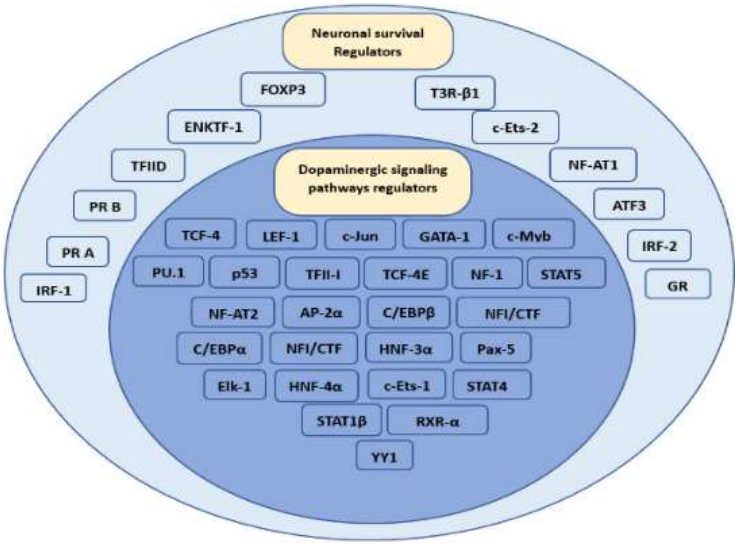


Figure 1: A schematic shows Regulators of neuronal function and dopaminergic signaling pathways as top TF candidates regulating ASCN gene involved in PD.

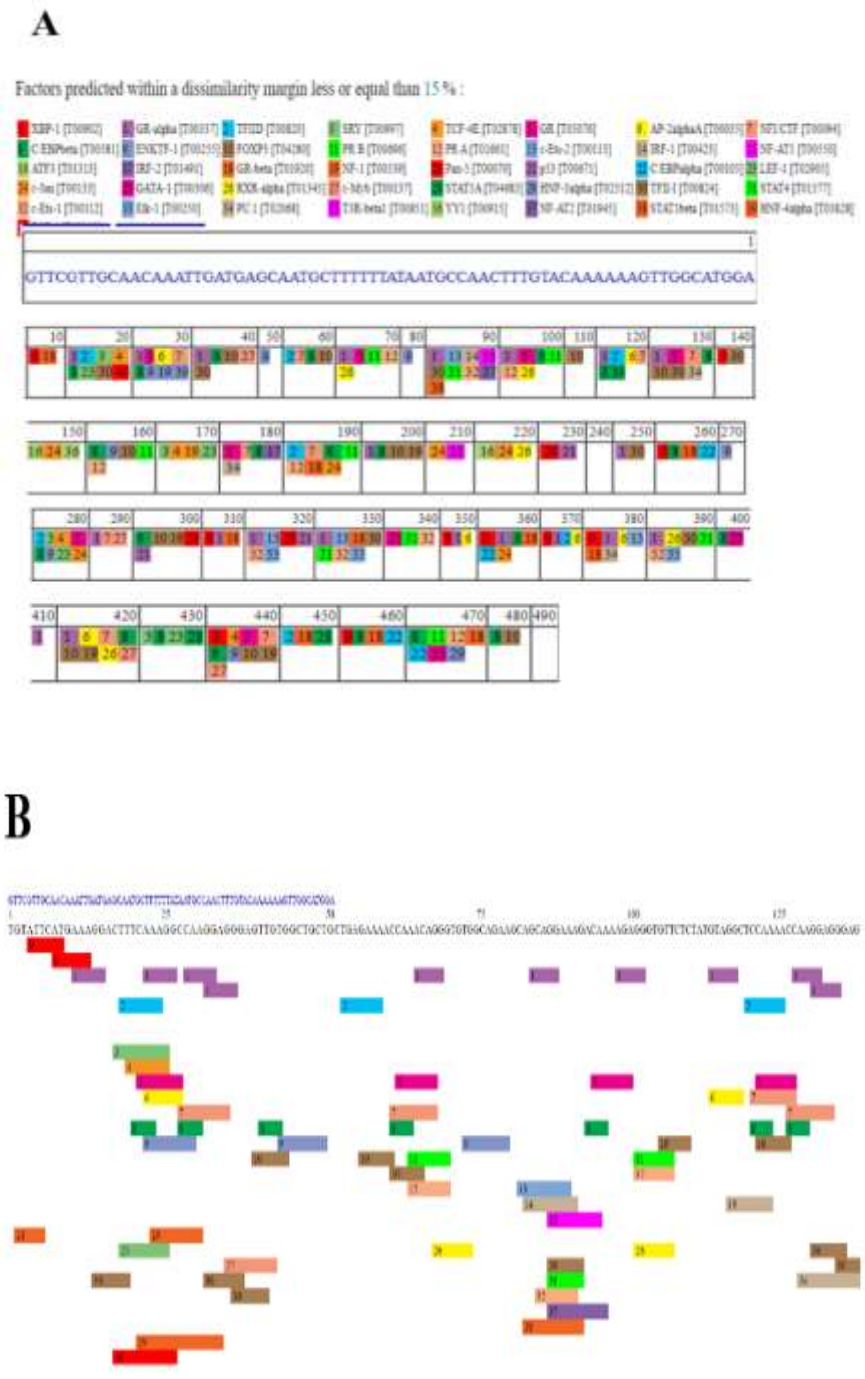
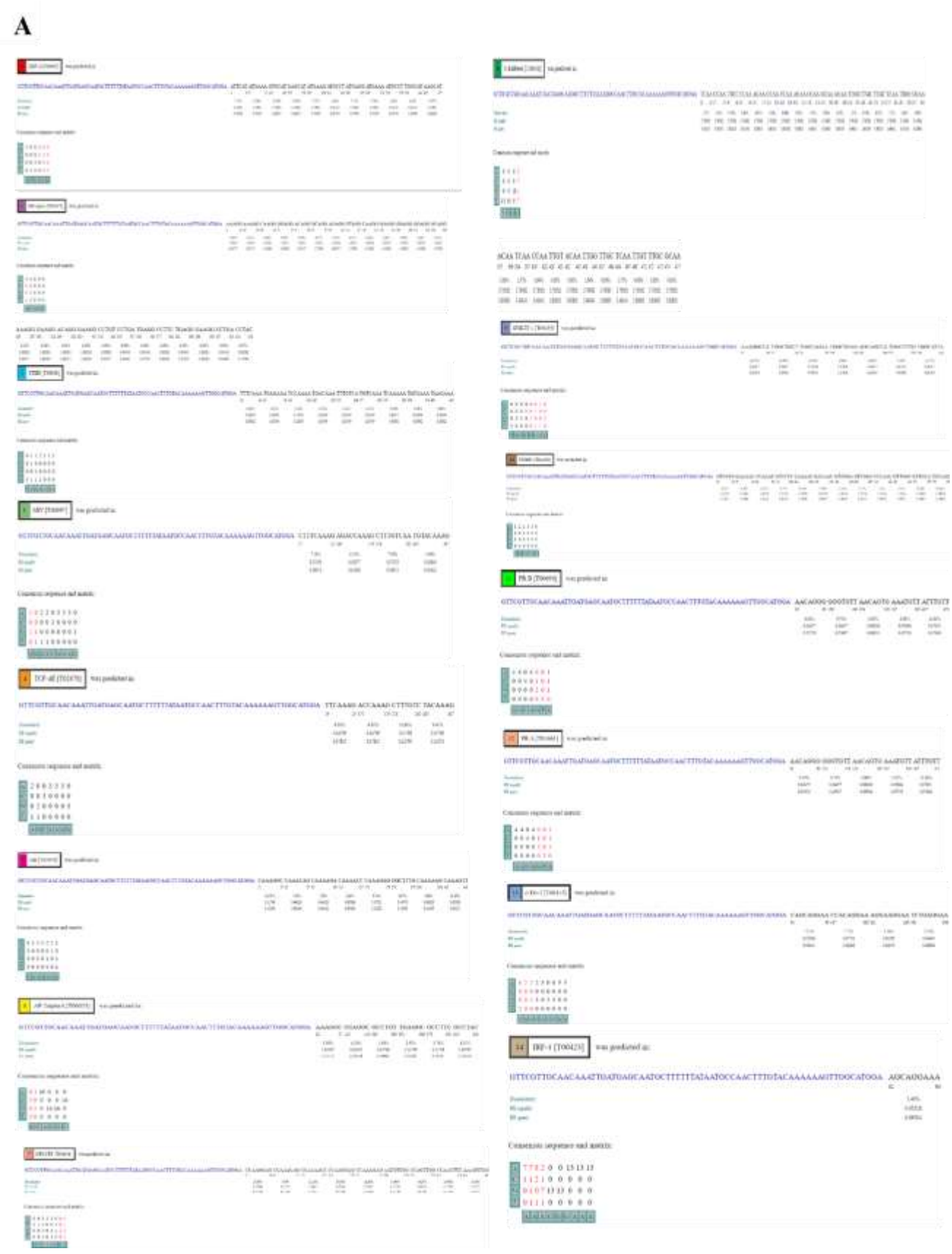


Figure 2: PROMO 'SearchSites' output. A. The regulatory region of human (SNCA)gene. Only those binding site predictions that appear in the sequences are shown, as boxes of different color and number. B. The image below, where the sequences are shown, is the result of selecting 'Zoom' in the main results page.


A


B

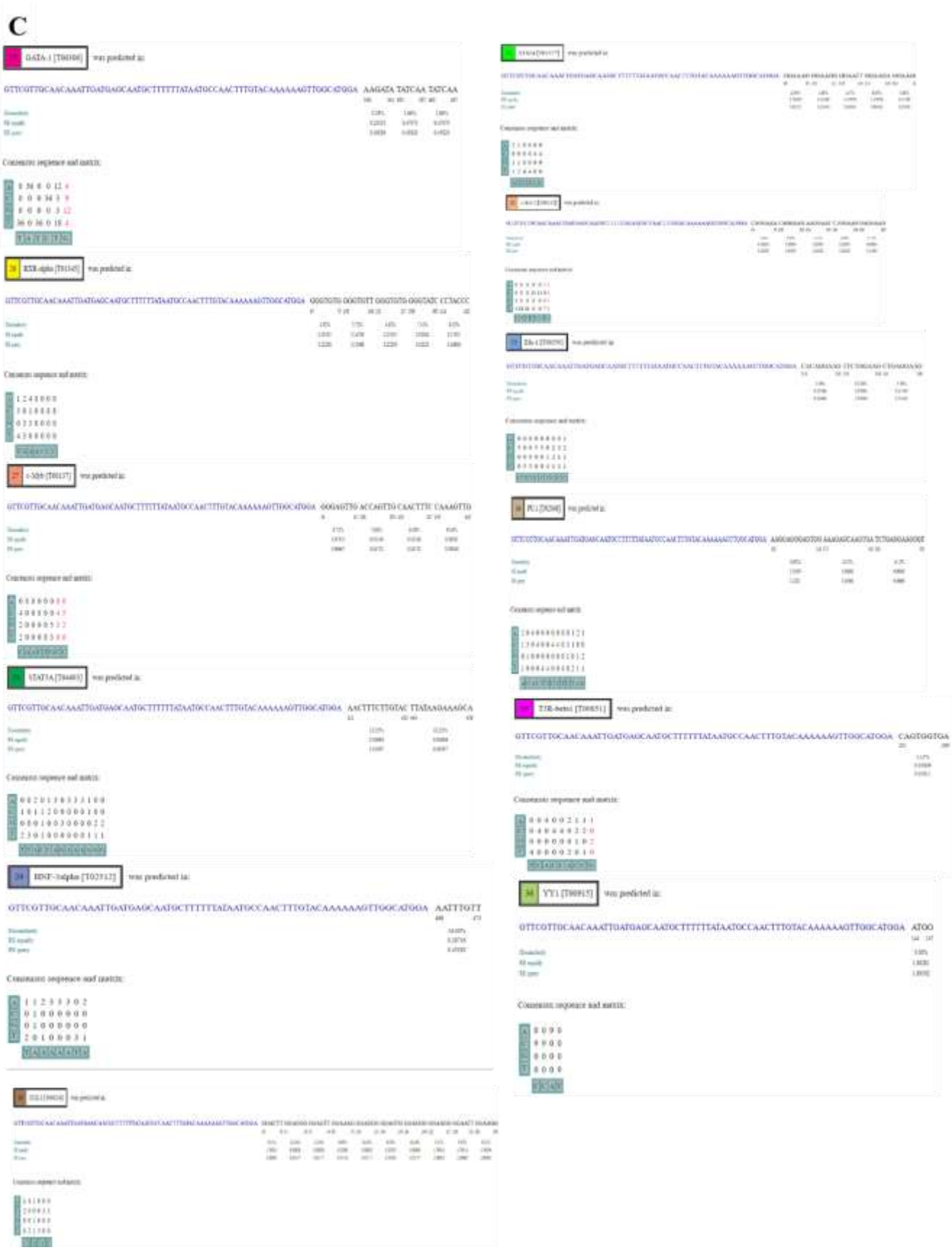

C

Figure 3: A-D, the details of the (SNCA) gene-bindings sites predictions on the sequences. They also show the weight matrix for the (SNCA) gene recognition sites and random expectations (RE) values for the different levels of sequence-matrix similarity. The RE is calculated with a model that considers that all nucleotides are equally probable and with a model that considers the nucleotide composition in the query sequence (in the picture represented by blue bars below matrix) (E).

4. Discussion

Predicting of transcription factor (TF) binding to genes involved in PD denotes a momentous step forward in unraveling the intricate regulatory mechanisms fundamental in disease pathogenesis [17,18]. Our study sheds light on the regulatory site of ASCN involved in PD by identifying putative TF-gene interactions and explaining their functional significance. Here, we investigate the contents of our findings, their significance to PD biology, and the potential translational implications for developing a therapy. One of the vital insights achieved from our predictive modeling is the identification of putative TFs that may play essential roles in governing PD-associated ASCN transcriptional dysregulation.

Utilizing computational analyses and applying multi-omics data, we revealed TFs with known involvement in neuronal function and dopaminergic signaling pathways. Among these, members of the Forkhead box (FOX) family and Nuclear Factor-kappa B (NF-κB that emerged as prominent regulators, proposing their possible contribution to PD pathophysiology figure 1.

Most TFs were those that play a crucial role in regulating gene expression in different cells including immune response, metabolism, cellular processes, and/or stress adaptation and apoptosis. These TFs cover C/EBPβ (CCAAT/enhancer-binding protein beta), CCAAT/enhancer-binding protein (SNCA)(C/EBPα), Interferon Regulatory Factor 2 (IRF-2), c-Jun, AP-2α, Interferon regulatory factor 1 (IRF-1), Nuclear Factor of Activated T cells 2 (NF-AT2), Activating Transcription Factor 3 (ATF3), Nuclear factor of activated T-cells 1 (NFAT1), Elk-1 (Ets Like-1), Yin Yang 1 (YY1), Forkhead Box A1 (FoxA1), c-MYB, the glucocorticoid receptor (SNCA)(GR-α-synuclein), Activating TFs such as ATF3 and TF4, in addition to signal transducers like STAT4 or STAT5A, all contribute in gene activation processes directing cell differentiation as they bind to specific promoters located within most genes' 5' regions [19-22]. Further developmental regulators that essentially act transiently at several stages include repressors such PAX5, which encodes a B-cell-specific activator protein (BSAP) [23], HNF3α (a protein linked with the differentiation of lung cells) [24], RXRα (a protein that cooperates with other nuclear receptors) or NFI, which is a transcription factor regularly originate in the brain. Despite frequent accounts around its role within cancer processes only growing evidence suggests otherwise its involvement in neurological disorders, including PD [25].

Specific proteins like FOXP3 participate in the formation and activation of T regulatory cells [26]. Moreover, TCF-4E and LEF- has been suggested to be involved in the Wnt signaling pathway whose other functions help in maintaining the normal tissues in developed organisms and early stages of existence or disorders such as those related to the brain or cancer [27]. P53 entails a paradigm of a cancer suppressor protein controlling gene restoration, aging process among other functions like cell cycle control [28]. While TFIID takes part in a series of reactions leading to formation of a certain substance [29]. PU.1 on the other hand is a key factor in hematopoiesis, the development of immune cells and in governing their

function [30]. Thyroid hormones (THs) control the gene transcription by TRβ1(nuclear receptor) [31]. In diverse regions including liver and pancreas HNF-4α belongs to superfamily of nuclear receptors controls expression patterns [32]. The SRY factor has been confirmed to play a major role by directing bipotential gonad differentiation leading to testis and eventually male sex determination [33]. STAT1β is a member of the STAT protein family that acts as a transcription factor in response to cytokines and growth factors [34]. While these proposed links endorse potential connections between the predicted TFs and PD, so far, there is no direct evidence of involvement of all these TFs in PD pathogenesis. Future work of experimental validation by TF-gene interactions through ChIP-seq and luciferase reporter assays will confirm their functional significance, providing mechanistic insights into the transcriptional regulation of PD-associated genes. Besides, additional studies investigating the denoted TFs roles in inflammation, oxidative stress, protein aggregation, and mitochondrial function in PD models may provide further visions into their implication in PD. These findings underscore the potential of predictive methods in elucidating disease mechanisms and identifying candidate therapeutic targets.

5. Conclusion

PD endures to present a challenge to the medical community, affecting millions worldwide with no cure in sight. However, the discovery of ASCN's key role has provided a deeper understanding of the disease's mechanisms. Unraveling the complexities of (SNCA) pathology holds the promise to emerging more effective treatments that could slow or halt PD progression. While the journey towards a cure remains laborious, the quest to decode the (SNCA) mystery offers hope for millions affected by this distressing neurological disorder. In this study, we exploited bioinformatics tools to predict the TFs that bind to the (SNCA) gene associated with PD. Knowing these TFs might explain the molecular based mechanisms behind it and determine potential therapeutic targets for PD. Future work will require a validation of these TF-gene interactions through ChIP-seq and luciferase reporter assays to confirm their functional implication, providing mechanistic insights into the transcriptional regulation of PD-associated genes.

6. References

1. Messeguer X., Escudero R., Dom D., Farré D., Farré F., Nez O., Martinez J., and M. Mar Alba., (2002). PROMO: detection of known transcription regulatory elements using species-tailored searches. Bioinformatics Applications Note. 18, 2, 333-334.
2. Wang Y., Zhao Y., Pan H., Zeng Q., Zhou X., Xiang Y., Zhou Z., Xu Q., Sun Q., Tan J., Yan X., Li J., Guo J., Tang B., Yu Q., and Liu Z., (2023). Genetic analysis of dystonia-related genes in Parkinson's disease. Front Aging Neurosci. 15, 1-11.
3. Narayan S., Liew Z., Bronstein J. M., and Ritz B., (2017). Occupational pesticide use and Parkinson's disease in the Parkinson Environment Gene (PEG) study. Environment international. 107, 266-273.
4. Calabresi P., Mechelli A., Natale G., Volpicelli-Daley L., Di Lazzaro G., and Ghiglieri V., (2023). Alpha-synuclein in Parkinson's disease and other synucleinopathies: from overt neurodegeneration back to early synaptic dysfunction. Cell Death and Disease. Springer Nature. 14, 176.
5. Gómez-Benito, M., Granado, N., García-Sanz, P., Michel, A., Dumoulin, M., and Moratalla, R., (2020). Modeling Parkinson's Disease With the Alpha-Synuclein Protein. Frontiers in pharmacology. 11, 356.
6. Leonidas Stefanis, (2012). α-Synuclein in Parkinson's disease. Cold Spring Harbor Perspectives in Medicine, 4, a009399.
7. Meade R. M., Fairlie D. P., and Mason J. M., (2019). Alpha-synuclein structure and Parkinson's disease - Lessons and emerging principles. Molecular Neurodegeneration. 14, 29.
8. Calabresi P., Di Lazzaro G., Marino G., Campanelli F., and Ghiglieri V., (2023). Advances in understanding the function of alpha-synuclein: implications for Parkinson's disease. Brain. 146, 9, 3587-3597.
9. Tonges L., and Zella M., (2019). Antibody-based immunotherapies for Parkinsonian syndromes. Neural Regeneration Research. 14, 1903-1904.
10. Zhao Y., Qin L., Pan H., Song T., Wang Y., Zhou X., Xiang Y., Li J., Liu Z., Sun Q., Guo J., Yan X., Tang B.,

and Xu Q., (2024). Genetic analysis of transcription factors in dopaminergic neuronal development in Parkinson's disease. Chinese medical journal. 137, 4, 450-456.

11. Tiwari C., Pal R., (2017). The potential role of neuroinflammation and transcription factors in Parkinson disease. Dialogues in Clinical Neuroscience. 19, 1, 71-80.
12. Farré D., Roset R., Huerta M., Adsuara J. E., Roselló L., Albà M. M., and Messeguer X. (2003). Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. Nucleic Acids Research. 31, 13, 3651-3653.
13. Hannenhalli S., and Levy S., (2002). Predicting transcription factor synergism. Nucleic Acids Research. 30, 19, 4278-4284.
14. Kaur S., Vashistt J., and Changotra H., (2024). Computational Investigation of Regulatory Region SNPs of Autophagy Gene BECN1. Defence Life Science Journal. 9, 1, 27-34.
15. Quandt K., Frech K., Karas H., Wingender E., and Werner T., (1995). MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucleic Acids Research. 23, 23, 4878-4884.
16. Martínez-Vicente P., Poblador F., Leitner J., Farré D., Steinberger P., Engel P., and Angulo A. (2022). Discovery of the first PD-1 ligand encoded by a pathogen. Frontiers in immunology, 13, 1007334.
17. Lambert S. A., Jolma A., Campitelli L. F., Das P. K., Yin Y., Albu M., Chen X., Taipale J., Hughes T. R., and Weirauch M. T., (2018). The Human Transcription Factors. Cell. 172, 4, 650-665.
18. Kim G. B., Gao, Y., Palsson, B. O., and Lee, S. Y. (2021). DeepTFactor: A deep learning-based tool for the prediction of transcription factors. Proceedings of the National Academy of Sciences of the United States of America. 118, 2, e2021171118.
19. Tanaka T., Yoshida N., Kishimoto T., and Akira S., (1997). Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. The EMBO journal. 16, 24, 7432-7443.
20. Koleva R. I., Ficarro S. B., Radomska H. S., Carrasco-Alfonso M. J., Alberta J. A., Webber J. T., Luckey C.

J., Marcucci G., Tenen D. G., and Marto J. A. (2012). C/EBPα and DEK coordinately regulate myeloid differentiation. Blood. 119, 21, 4878-4888.

21. Persyn E., Wahlen S., Kiekens L., Van Loocke W., Siwe H., Van Ammel E., De Vos Z., Van Nieuwerburgh F., Matthys P., Taghon T., Vandekerckhove B., Van Vlierberghe P., and Leclercq G., (2022). IRF2 is required for development and functional maturation of human NK cells. Frontiers in immunology. 13, 1038821.
22. Meng Q., and Xia, Y. (2011). c-Jun, at the crossroad of the signaling network. Protein and cell. 2, 11, 889-898.
23. McManus S., Ebert A., Salvagiotto G., Medvedovic J., Sun Q., Tamir I., Jaritz M., Tagoh H., and Busslinger M., (2011). The transcription factor Pax5 regulates its target genes by recruiting chromatin-modifying proteins in committed B cells. The EMBO journal. 30, 12, 2388-2404.
24. Klaus H Kaestner., (2000). The Hepatocyte Nuclear Factor 3 (HNF3 or FOXA) Family in Metabolism, Trends in Endocrinology and Metabolism. 11, 7, 281-285.
25. Zati Zehni A, Batz F, Cavaillès V, Sixou S, Kaltofen T, Keckstein S, Helene Hildegard Heidegger, Ditsch N., Mahner S., Jeschke U., and Vilsmaier T., (2021). Cytoplasmic localization of rxrα determines outcome in breast cancer. Cancers (Basel). 13, 15, 3756.
26. Frattini V., Pisati F., Speranza M. C., Poliani P. L., Frigé G., Cantini G., Kapetis D., Cominelli M., Rossi A., Finocchiaro G., and Pellegatta S., (2012). FOXP3, a novel glioblastoma oncosuppressor, affects proliferation and migration. Oncotarget. 3, 10, 1146-1157.
27. Hrckulak D., Janeckova L., Lanikova L., Kriz V., Horazna M., Babosova O., Vojtechova M., Galuskova K., Sloncova E., and Korinek V., (2018). Wnt Effector TCF4 Is Dispensable for Wnt Signaling in Human Cancer Cells. Genes. 9, 9, 439.
28. Reisman D., Takahashi P., Polson A., and Boggs K., (2012). Transcriptional Regulation of the p53 Tumor Suppressor Gene in S-Phase of the Cell-Cycle and the Cellular Response

to DNA Damage. Biochemistry research international. 808934.

29. Patel A. B., Greber B. J., and Nogales E., (2020). Recent insights into the structure of TFIID, its assembly, and its binding to core promoter. Current opinion in structural biology, 61, 17-24.
30. Rothenberg E V., Hosokawa H, and Ungerbäck J. (2019). Mechanisms of action of hematopoietic transcription factor PU.1 in initiation of T-cell development. Front Immunol. 10, 228.
31. Asghar M. Y., Knuutinen T., Holm E., Nordström T., Nguyen V. D., Zhou Y., and Törnquist K. (2022). Suppression of Calcium Entry Modulates the Expression of TRβ1 and Runx2 in Thyroid Cancer Cells, Two Transcription Factors That Regulate Invasion, Proliferation and Thyroid-Specific Protein Levels. Cancers, 14(23), 5838.
32. Walesky C., and Apte U., (2015). Role of hepatocyte nuclear factor 4α (HNF4α) in cell proliferation and cancer. Gene expression. 16, 3, 101-108.
33. Matsuzawa-Watanabe Y., Inoue J. I., and Semba K., (2003). Transcriptional activity of testis-determining factor SRY is modulated by the Wilms' tumor 1 gene product, WT1. Oncogene. 22, 39, 7900-7904.
34. Semper C., Leitner N. R., Lassnig C., Parrini M., Mahlakõiv T., Rammerstorfer M., Lorenz K., Rigler D., Müller S., Kolbe T., Vogl C., Rülicke T., Staeheli P., Decker T., Müller M., and Strobl B. (2014). STAT1β is not dominant negative and is capable of contributing to gamma interferon-dependent innate immunity. Molecular and cellular biology. 34, 12, 2235-2248.