SNCA and PTPRK are promising druggable targets for treating Parkinson Disease that control activity of HLTF, POU2F1 and RELA transcription factors on promoters of differentially expressed genes

Demo User
geneXplain GmbH
info@genexplain.com
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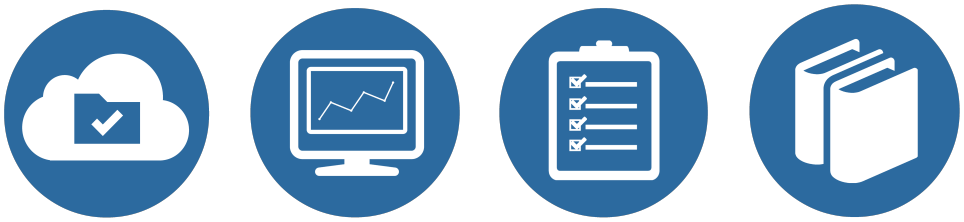
Genome Enhancer release 3.4 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2024.1)



Abstract

In the present study we applied the software package "Genome Enhancer" to a data set that contains *transcriptomics* data. The study is done in the context of *Parkinson Disease*. The goal of this pipeline is to identify potential drug targets in the molecular network that governs the studied pathological process. In the first step of analysis pipeline discovers transcription factors (TFs) that regulate genes activities in the pathological state. The activities of these TFs are controlled by so-called master regulators, which are identified in the second step of analysis. After a subsequent druggability checkup, the most promising master regulators are chosen as potential drug targets for the analyzed pathology. At the end the pipeline comes up with (a) a list of known drugs and (b) investigational active chemical compounds with the potential to interact with selected drug targets.

From the data set analyzed in this study, we found the following TFs to be potentially involved in the regulation of the differentially expressed genes: HLTF, POU2F1, THRB, RELA, GCM1 and MAFG. The subsequent network analysis suggested

- Caspase-8
- TC-PTP
- SNCA

as the most promising molecular targets for further research, drug development and drug repurposing initiatives on the basis of identified molecular mechanism of the studied pathology. Having checked the actual druggability potential of the full list of identified targets, both, via information available in medical literature and via cheminformatics analysis of drug compounds, we have identified the following drugs as the most promising treatment candidates for the studied pathology: Sirolimus, 1-(5-Tert-Butyl-2-P-Tolyl-2h-Pyrazol-3-Yl)-3-[4-(2-Morpholin-4-Yl-Ethoxy)-Naphthalen-1-Yl]-Urea, Lipoic Acid and 2,5,7-Trihydroxynaphthoquinone.

1. Introduction

Recording "-omics" data to measure gene activities, protein expression or metabolic events is becoming a standard approach to characterize the pathological state of an affected organism or tissue. Increasingly, several of these methods are applied in a combined approach leading to large "multiomics" datasets. Still the challenge remains how to reveal the underlying molecular mechanisms that render a given pathological state different from the norm. The disease-causing mechanism can be described by a re-wiring of the cellular regulatory network, for instance as a result of a genetic or epigenetic alterations influencing the activity of relevant genes. Reconstruction of the disease-specific regulatory networks can help identify potential master regulators of the respective pathological process. Knowledge about these master regulators can point to ways how to block a pathological regulatory cascade. Suppression of certain molecular targets as components of these cascades may stop the pathological process and cure the disease.

Conventional approaches of statistical "-omics" data analysis provide only very limited information about the causes of the observed phenomena and therefore contribute little to the understanding of the pathological molecular mechanism. In contrast, the "upstream analysis" method [1-4] applied here has been deviced to provide a casual interpretation of the data obtained for a pathology state. This approach comprises two major steps: (1) analysing promoters and enhancers of differentially expressed genes for the transcription factors (TFs) involved in their regulation and, thus, important for the process under study; (2) re-constructing the signaling pathways that activate these TFs and identifying master regulators at the top of such pathways. For the first step, the database TRANSFAC® [6] is employed together with the TF binding site identification algorithms Match [7] and CMA [8]. The second step involves the signal transduction database TRANSPATH® [9] and special graph search algorithms [10] implemented in the software "Genome Enhancer".

The "upstream analysis" approach has now been extended by a third step that reveals known drugs suitable to inhibit (or activate) the identified molecular targets in the context of the disease under study. This step is performed by using information from HumanPSD™ database [5]. In addition, some known drugs and investigational active chemical compounds are subsequently predicted as potential ligands for the revealed molecular targets. They are predicted using a pre-computed database of spectra of biological activities of chemical compounds of a library of 2245 known drugs and investigational chemical compounds from HumanPSD™ database. The spectra of biological activities for these compounds are computed using the program PASS on the basis of a (Q)SAR approach [11-13]. These predictions can be used for the research purposes - for further drug development and drug repurposing initiatives.

2. Data

For this study the following experimental data was used:

Table 1. Experimental datasets used in the study

File name	Data type
GSE145804_DESeq2_final	Transcriptomics

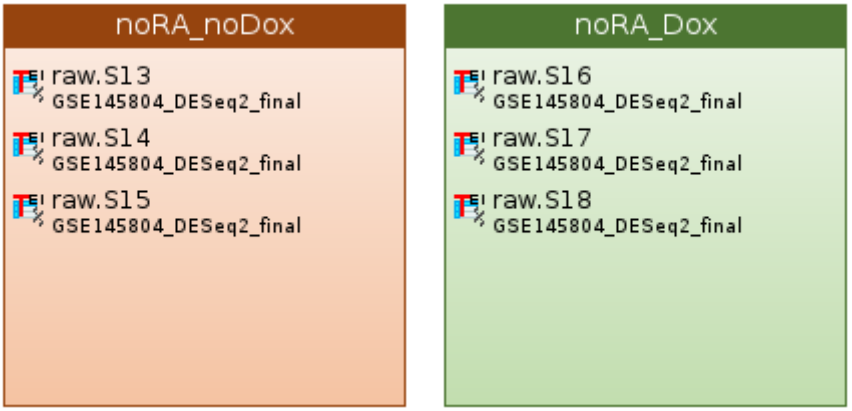


Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.

3. Results

We have compared the following conditions: noRA_Dox *versus* noRA_noDox.

3.1. Identification of target genes

In the first step of the analysis ***target genes*** were identified from the uploaded experimental data. We applied the edgeR tool (R/Bioconductor package integrated into our pipeline) and compared gene expression in the following sets: "noRA_Dox" with "noRA_noDox". edgeR calculated the LogFC (the logarithm to the base 2 of the fold change between different conditions), the p-value and the adjusted p-value (corrected for multiple testing) of the observed fold change. As a result, we detected 3391 upregulated genes (LogFC>0.1) out of which 333 genes were found as significantly upregulated (p-value<0.1) and 4391 downregulated genes (LogFC<-0.1) out of which 344 genes were significantly downregulated (p-value<0.1). See tables below for the top significantly up- and downregulated genes. Below we call **target genes** the full list of up- and downregulated genes revealed in our analysis (see tables in Supplementary section).

*Table 2. Top ten significant **up-regulated** genes in noRA_Dox vs. noRA_noDox.*
See full table →

ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000145335	SNCA	synuclein alpha	4.38	10.37	5.54E-90	6.59E-86
ENSG00000206651	Y_RNA	Y RNA	1.27	2.08	8.17E-2	0.96
ENSG00000108231	LGI1	leucine rich glioma inactivated 1	1.04	3.42	4.04E-3	0.96
ENSG00000205403	CFI	complement factor I	1.03	3.11	1.25E-4	0.17
ENSG00000275140	SEC22B3P	SEC22 homolog B3, pseudogene	1.03	2.97	8.86E-3	0.96
ENSG00000189057	FAM111B	FAM111 trypsin like peptidase B	0.95	3.47	1.18E-3	0.56
ENSG00000231043		IK cytokine, down-regulator of HLA II (IK) pseudogene	0.95	2.41	2.56E-2	0.96
ENSG00000108691	CCL2	C-C motif chemokine ligand 2	0.94	4.19	5.39E-6	1.07E-2
ENSG00000086300	SNX10	sorting nexin 10	0.94	3.01	4.34E-4	0.36
ENSG00000265972	TXNIP	thioredoxin interacting protein	0.87	8.05	7.68E-10	3.04E-6

*Table 3. Top ten significant **down-regulated** genes in noRA_Dox vs. noRA_noDox.*
See full table →

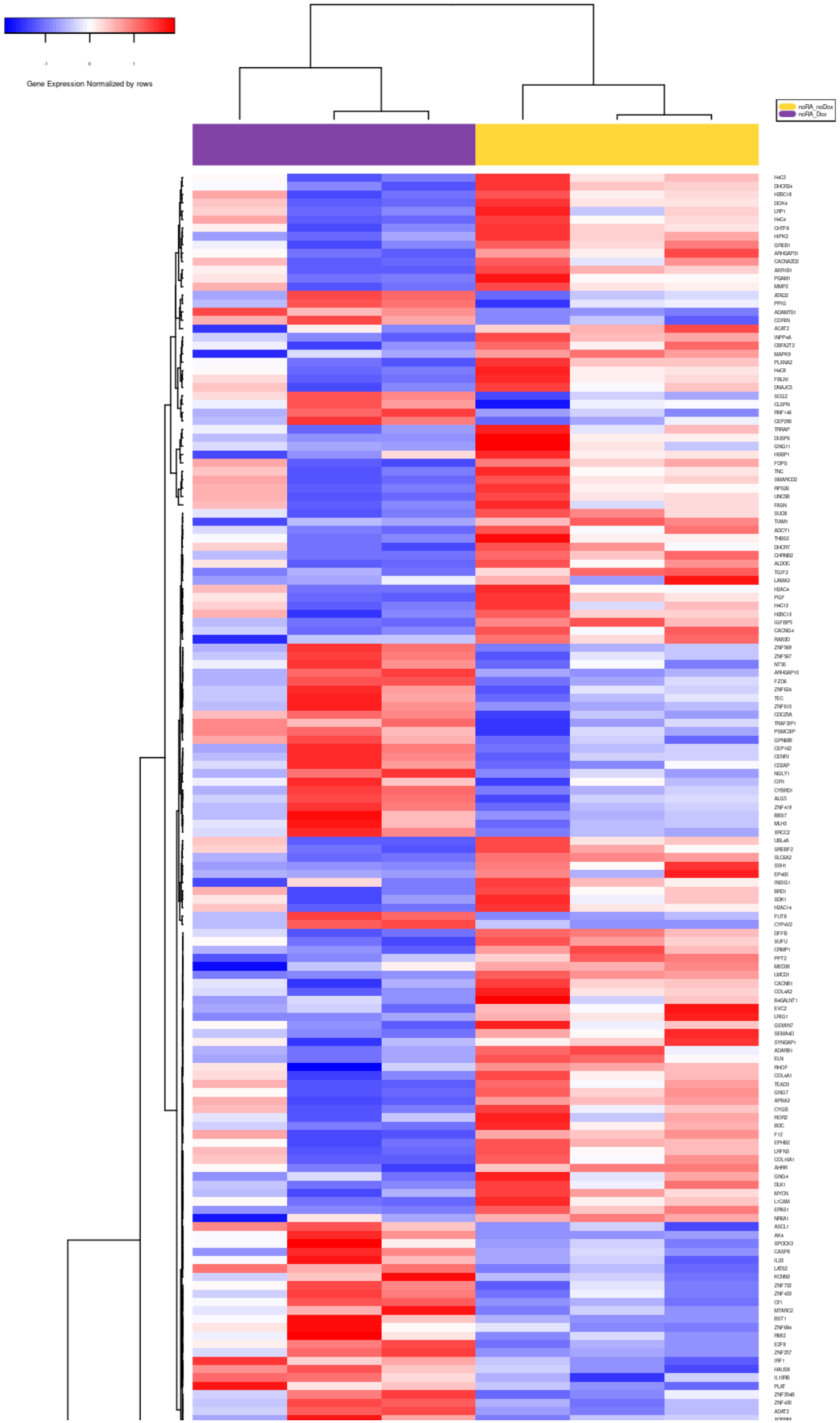
ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000186081	KRT5	keratin 5	-10.37	2.59	4.32E-6	1.03E-2
ENSG00000147256	ARHGAP36	Rho GTPase activating protein 36	-1.67	5.58	3.6E-16	2.14E-12
ENSG00000157601	MX1	MX dynamin like GTPase 1	-1.64	1.75	1.44E-2	0.96
ENSG00000123454	DBH	dopamine beta-hydroxylase	-1.48	3.54	4.57E-5	6.78E-2
ENSG00000254656	RTL1	retrotransposon Gag like 1	-1.36	3.09	2.62E-4	0.31
ENSG00000116016	EPAS1	endothelial PAS domain protein 1	-1.29	2.34	2.42E-6	7.19E-3
ENSG00000185559	DLK1	delta like non-canonical Notch ligand 1	-1.15	2.69	8.96E-4	0.48
ENSG00000165912	PACSIN3	protein kinase C and casein kinase substrate in neurons 3	-1.05	3.21	1.55E-2	0.96
ENSG00000148357	HMCN2	hemicentin 2	-1.03	2.42	1.01E-2	0.96
ENSG00000049540	ELN	elastin	-0.95	2.92	5.79E-4	0.36

3.2. Functional classification of genes

A functional analysis of differentially expressed genes was done by mapping the significant up-regulated and significant down-regulated genes to several known ontologies, such as Gene Ontology (GO), disease ontology (based on HumanPSD™ database) and the ontology of signal transduction and metabolic pathways from the TRANSPATH® database. Statistical significance was computed using a binomial test.
Figures 3-8 show the most significant categories.

Heatmap of differentially expressed genes in noRA_Dox vs. noRA_noDox

A heatmap of all differentially expressed genes playing a potential regulatory role in the system (enriched in TRANSPATH® pathways) is presented in Figure 2.


-1
0
1
Gene Expression Normalized by rows
noRA_noDox
noRA_Dox
H4C3
DHCR24
H2BC18
DOK4
LRP1
H4C4
CHTF8
HIPK2
GREB1
ARHGAP31
CACNA2D2
AKR1B1
PGAM1
MMP2
ATAD2
PPIG
ADAMTS1
CORIN
AGAT2
INPP4A
CBFA2T2
MAPK9
PLXNA2
H4C8
FBLN1
DNAJC5
SOG2
CLSPN
RNF146
CEP290
TRRAP
DUSP6
GNG11
HSBP1
FDPS
TNC
SMARCD2
RPS26
UNC5B
FASN
SUOX
TIAM1
ADCY1
THBS2
DHCR7
CHFR62
ALDOC
TGIF2
LAMA3
H2AC4
PGF
H4C13
H2BC13
IGFBP5
CACNG4
RAB3D
ZNF569
ZNF567
NT5E
ARHGAP10
FZD6
ZNF624
TEC
ZNF810
CDC25A
TRAF3IP1
PSMC3IP
GPNMB
CEP162
CENPJ
CDDAP
NGLY1
CIR1
CYBRD1
ALG5
ZNF419
BBS7
MUH3
XRCC2
UBL4A
SREBF2
SLC6A2
SSH1
EP400
INSIG1
BRD1
SDK1
H2AC14
FUT8
CYP4V2
DFFB
SUFU
CRIMP1
PPT2
MED30
LMCD1
CACNB1
COL4A2
B4GALNT1
EVC2
LRIG1
GEMIN7
SEMA4D
SYNGAP1
ADARB1
ELN
RHOF
COL4A1
TEAD3
GNG7
APBA3
CYGB
ROR2
BOC
F12
EPHB2
LRFN3
COL16A1
AHRR
GNG4
DLK1
MYCN
L1CAM
ERAS1
NR6A1
ASCL1
AK4
SPOCK3
CASP8
IL33
LATS2
KCNN3
ZNF732
ZNF430
CFI
MTARC2
BST1
ZNF684
RMI2
E2F8
ZNF257
IRF1
HAUS8
IL10RB
PLAT
ZNF354B
ZNF430
ADAT2

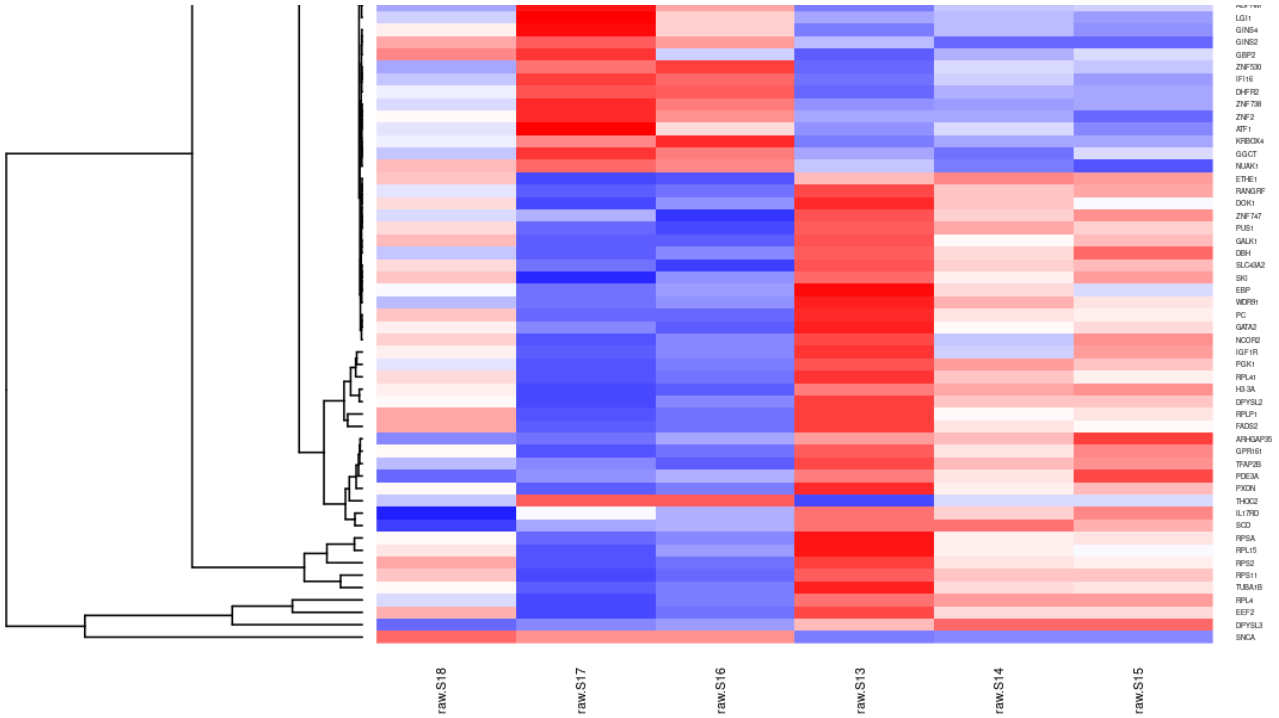


Figure 2. Heatmap of genes enriched in Transpath categories. The colored bar at the top shows the types of the samples according to the legend in the upper right corner.

See full diagram →

Up-regulated genes in noRA_Dox vs. noRA_noDox:

333 significant up-regulated genes were taken for the mapping.

GO (biological process)

Figure 3. Enriched GO (biological process) of up-regulated genes in noRA_Dox vs. noRA_noDox.

Full classification →

TRANSPATH® Pathways (2024.1)

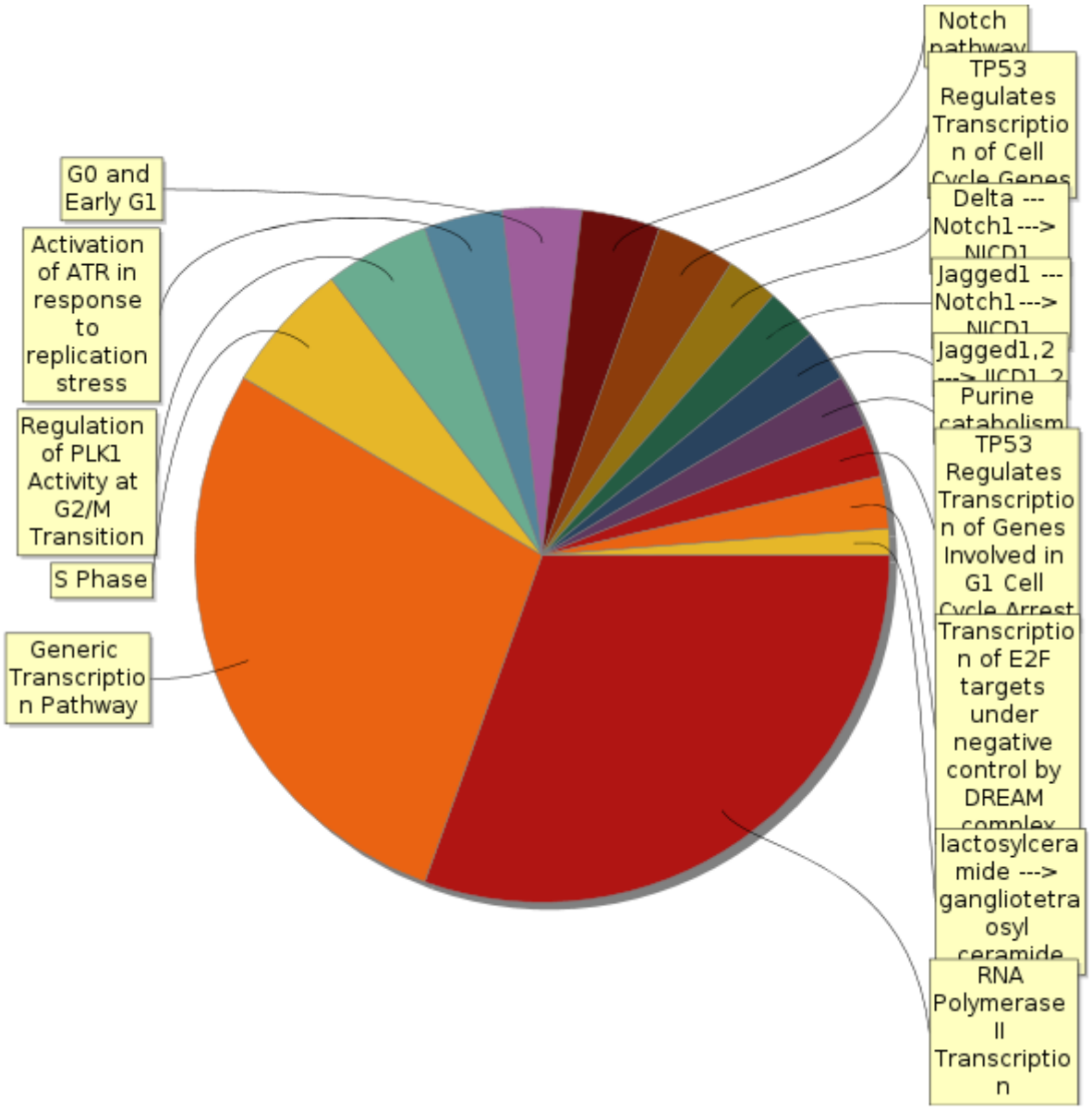


Figure 4. Enriched TRANSPATH® Pathways (2024.1) of up-regulated genes in noRA_Dox vs. noRA_noDox.

Full classification →

HumanPSD(TM) disease (2024.1)

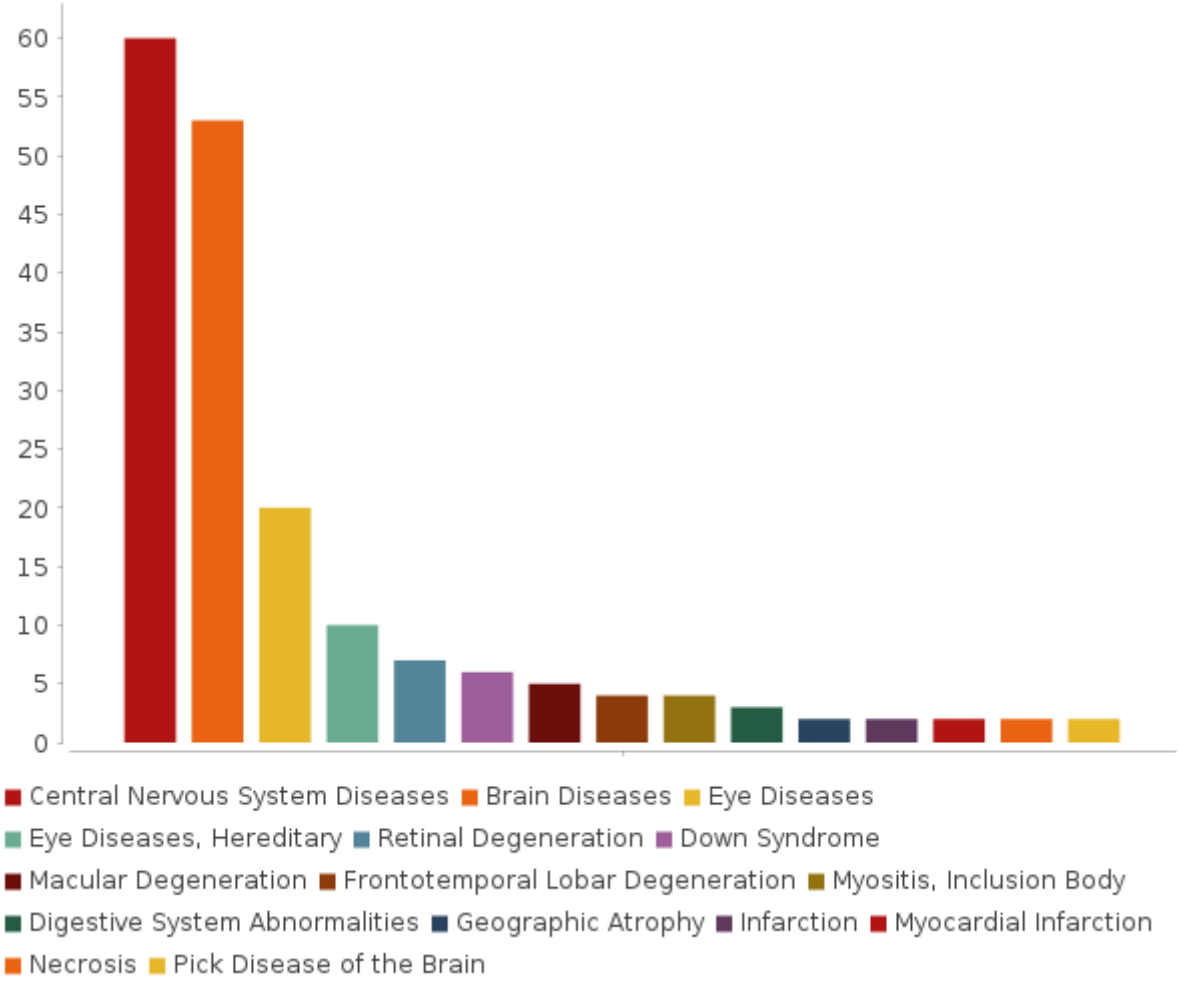


Figure 5. Enriched HumanPSD(TM) disease (2024.1) of up-regulated genes in noRA_Dox vs. noRA_noDox. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.

Full classification →

Down-regulated genes in noRA_Dox vs. noRA_noDox:

344 significant down-regulated genes were taken for the mapping.

GO (biological process)

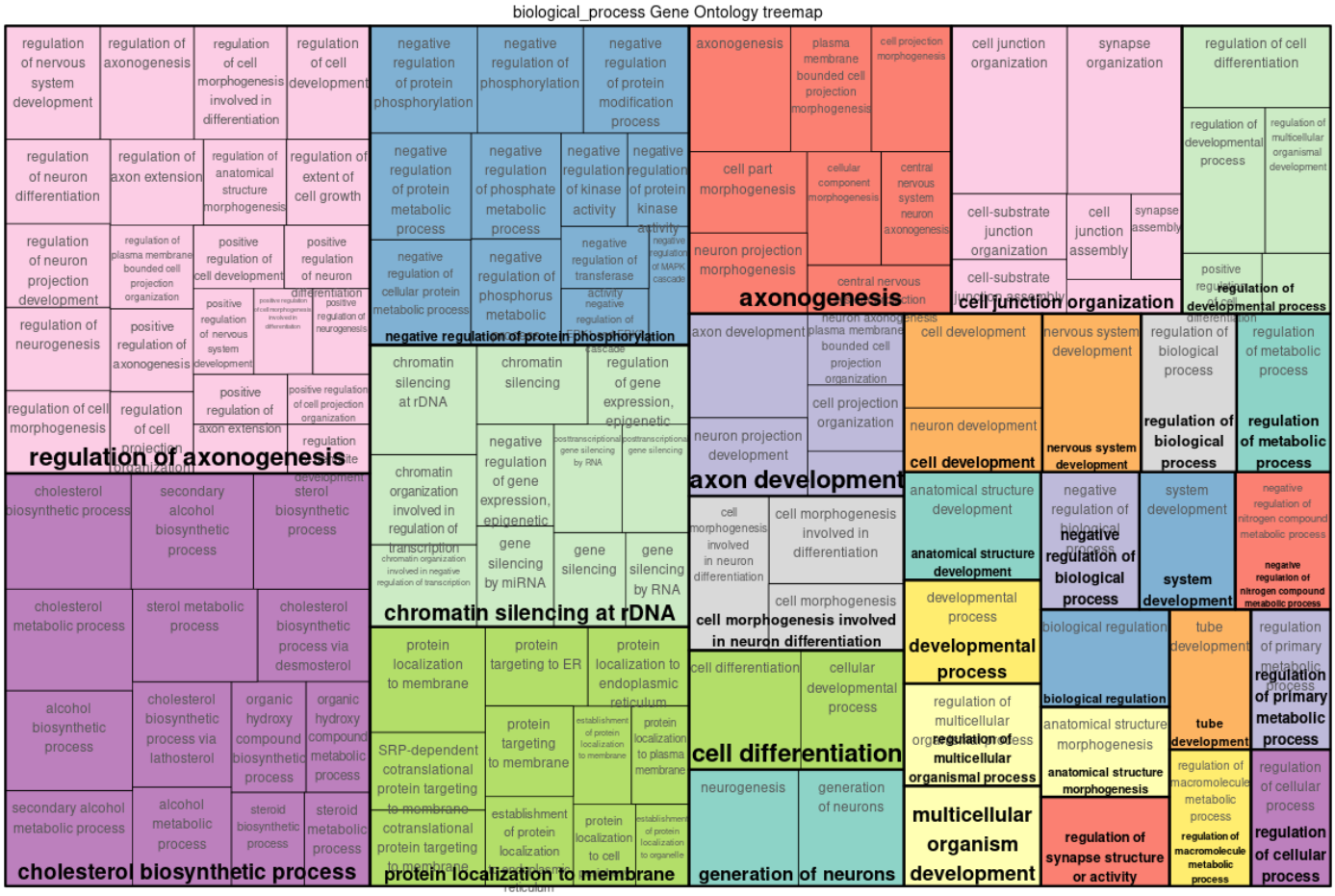


Figure 6. Enriched GO (biological process) of down-regulated genes in noRA_Dox vs. noRA_noDox.

Full classification →

TRANSPATH® Pathways (2024.1)

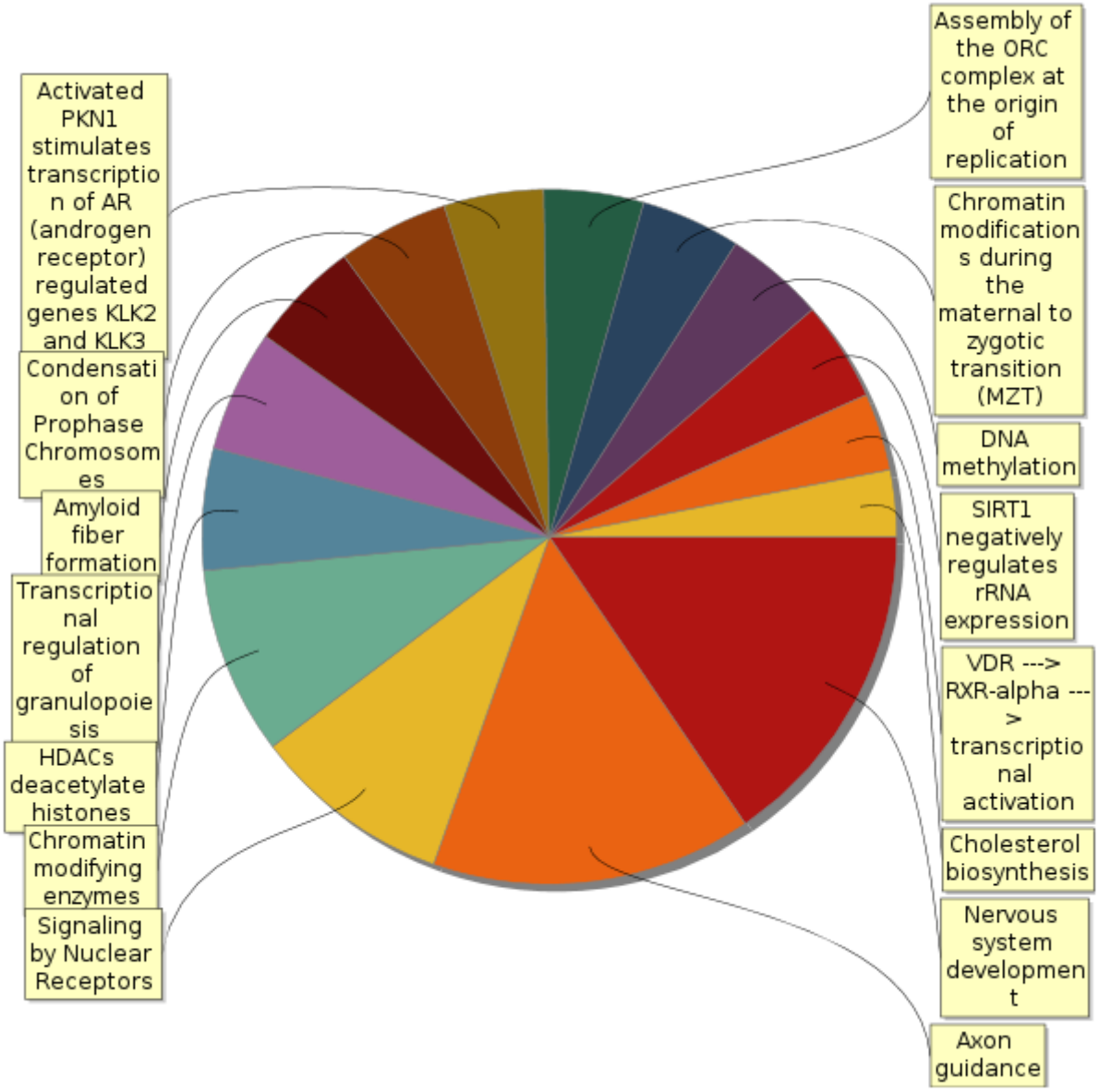


Figure 7. Enriched TRANSPATH® Pathways (2024.1) of down-regulated genes in noRA_Dox vs. noRA_noDox.
Full classification →

HumanPSD(TM) disease (2024.1)

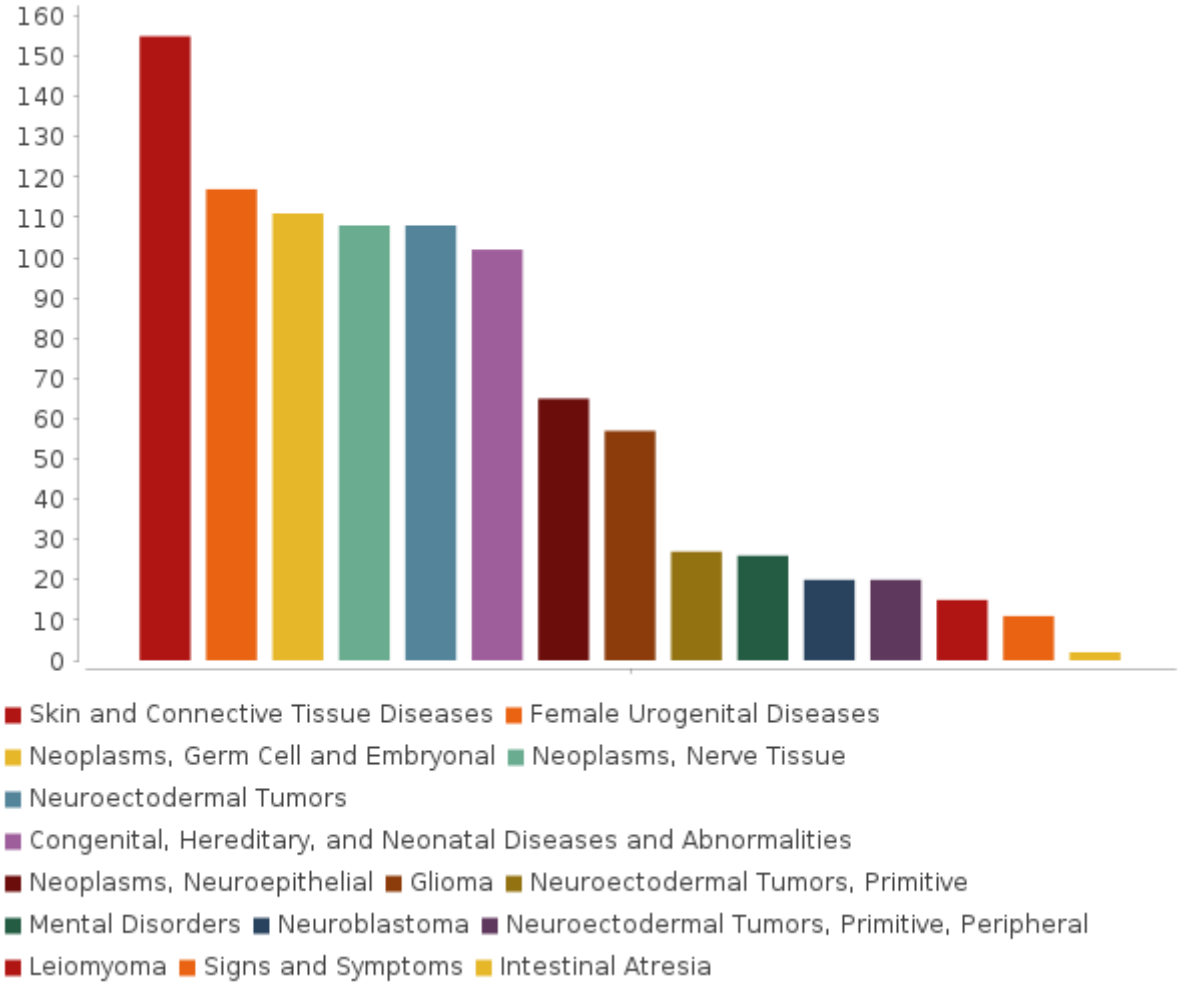
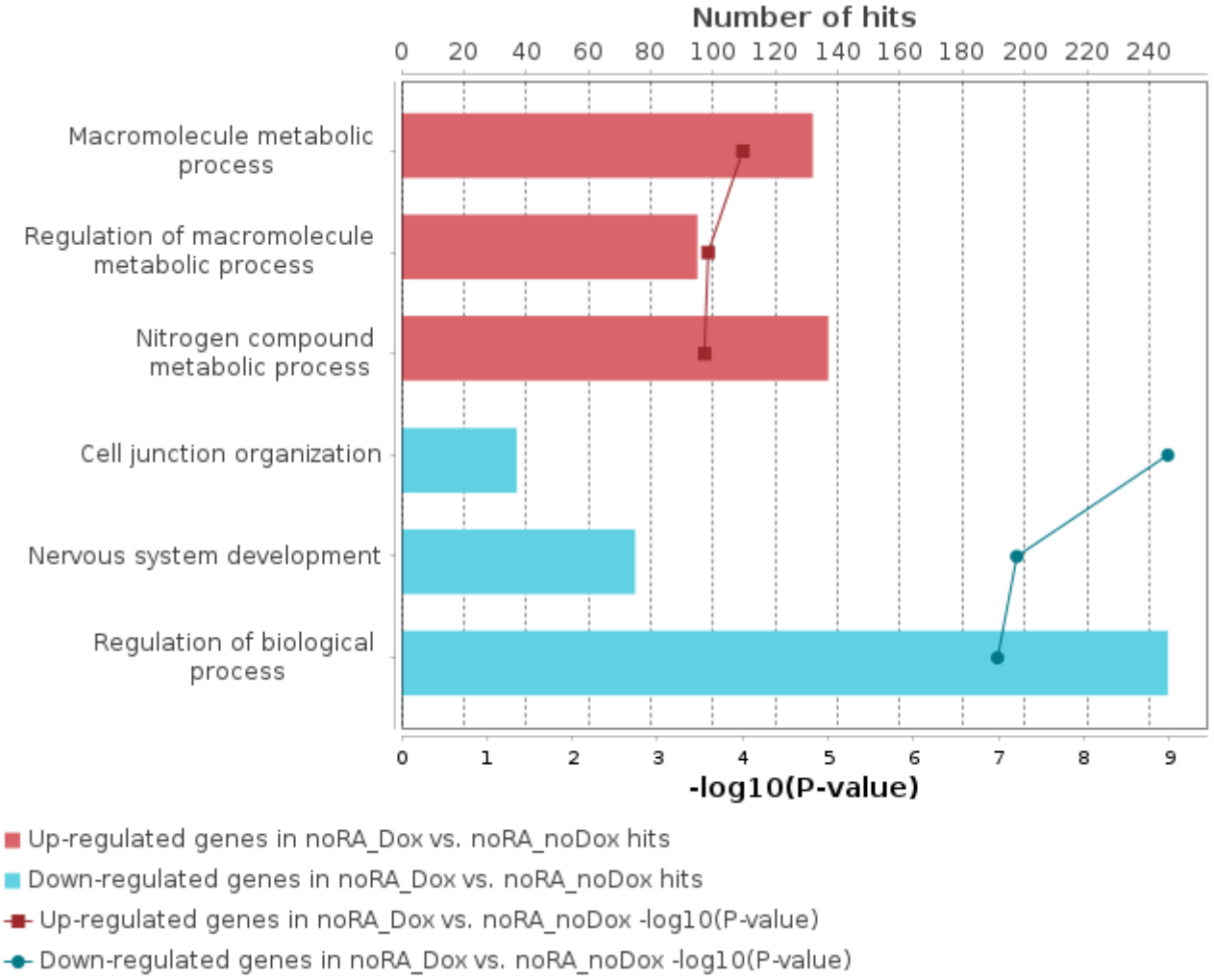


Figure 8. Enriched HumanPSD(TM) disease (2024.1) of down-regulated genes in noRA_Dox vs. noRA_noDox. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.

Full classification →

The result of overall Gene Ontology (GO) analysis of the differentially expressed genes of the studied pathology can be summarized by the following diagram, revealing the most significant functional categories overrepresented among the observed (differentially expressed genes):



3.3. Analysis of enriched transcription factor binding sites and composite modules

In the next step a search for transcription factors binding sites (TFBS) was performed in the regulatory regions of the ***target genes*** by using the TF binding motif library of the TRANSFAC® database. We searched for so called **composite modules** that act as potential condition-specific **enhancers** of the ***target genes*** in their upstream regulatory regions (-1000 bp upstream of transcription start site (TSS)) and identify transcription factors regulating activity of the genes through such **enhancers**.

Classically, **enhancers** are defined as regions in the genome that increase transcription of one or several genes when inserted in either orientation at various distances upstream or downstream of the gene [8]. Enhancers typically have a length of several hundreds of nucleotides and are bound by multiple transcription factors in a cooperative manner [9].

We applied the Composite Module Analyst (CMA) [8] method to detect such potential enhancers, as targets of multiple TFs bound in a cooperative manner to the regulatory regions of the genes of interest. CMA applies a genetic algorithm to construct a generalized model of the enhancers by specifying combinations of TF motifs (from TRANSFAC®) whose sites are most frequently clustered together in the regulatory regions of the studied genes. CMA identifies the transcription factors that through their cooperation provide a synergistic effect and thus have a great influence on the gene regulation process.

Enhancer model potentially involved in regulation of target genes (up-regulated genes in noRA_Dox vs. noRA_noDox).

To build the most specific composite modules we choose top 300 significant up-regulated genes as the input of CMA algorithm. The obtained CMA model is then applied to compute CMA score for all up-regulated genes in noRA_Dox vs. noRA_noDox.

The model consists of 2 module(s). Below, for each module the following information is shown:
- PWMs producing matches,
- number of individual matches for each PWM,
- score of the best match.

Module 1:

V$POU1F1_Q6	V$HMEF2_Q6	V$THRB_10	V$SREBP_Q3	V$MAFK_07
0.87; N=2	0.76; N=3	0.81; N=2	0.93; N=3	0.79; N=2

Module width: 141

Module 2:

V$PBX1_13	V$OCT1_B	V$RUSH1A_02	V$FOXG1_08	V$HOXA10_Q6
0.85; N=1	0.82; N=2	0.98; N=2	0.96; N=3	0.99; N=2

Module width: 115

Model score (-p*log10(pval)): 14.12
Wilcoxon p-value (pval): 6.67e-29
Penalty (p): 0.501
Average yes-set score: 5.24
Average no-set score: 3.66
AUC: 0.73
Separation point: 5.09
False-positive: 22.65%
False-negative: 40.67%

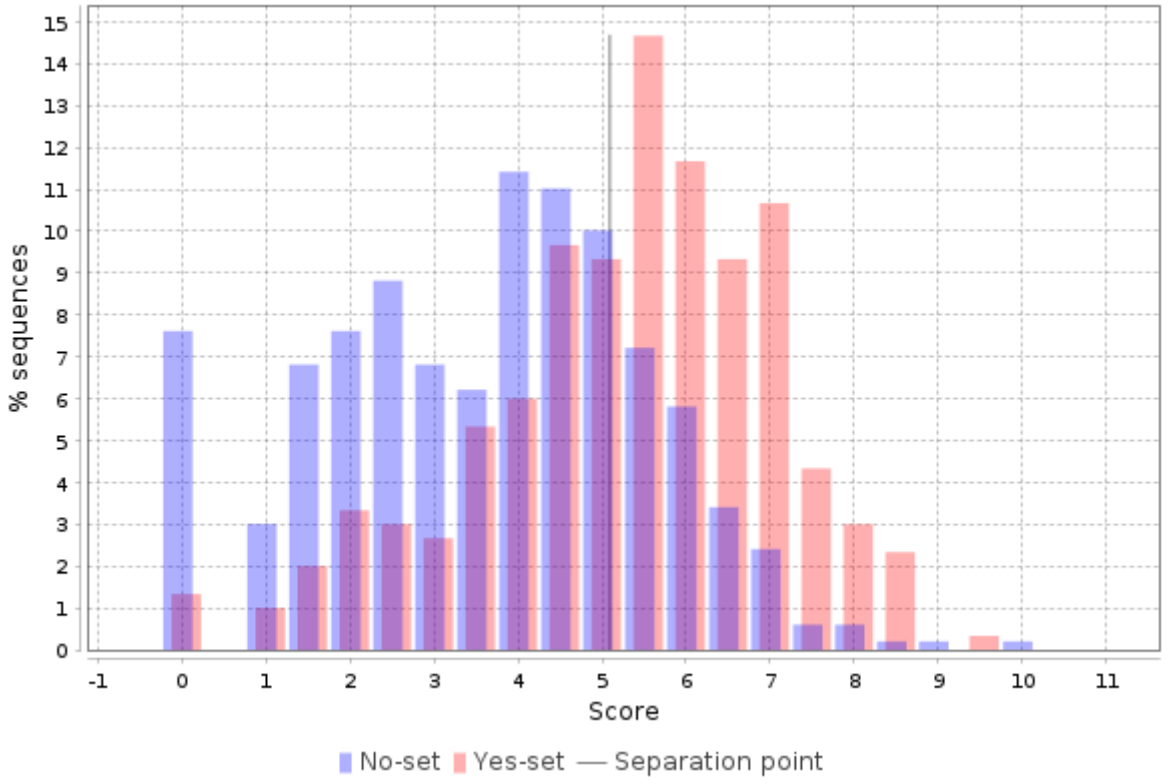


Table 4. List of top ten up-regulated genes in noRA_Dox vs. noRA_noDox with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000163075	CFAP221	cilia and flagella associated protein 221	11	POU1F1(h), MafK(h), PBX-1(h), POU2F1(h), MEF-2A(h), Hox-A10(h), HLTF(h)
ENSG00000069849	ATP1B3	ATPase Na+/K+ transporting subunit beta 3	10.19	MEF-2A(h), Hox-A10(h), HLTF(h), POU1F1(h), POU2F1(h), MafK(h), SREBP-1(h),SREBP-2(h)...
ENSG00000121058	COIL	coilin	9.56	T3R-beta(h), POU1F1(h), MEF-2A(h), MafK(h), Hox-A10(h), POU2F1(h), HLTF(h)
ENSG00000197223	C1D	C1D nuclear receptor corepressor	9.53	FOXG1(h), MEF-2A(h), HLTF(h), POU2F1(h), Hox-A10(h), MafK(h), POU1F1(h)...
ENSG00000283443		zinc finger protein 285 (ZNF285) pseudogene	9.36	MEF-2A(h), MafK(h), POU1F1(h), T3R-beta(h), POU2F1(h), HLTF(h), Hox-A10(h)...
ENSG00000229638	RPL4P4	ribosomal protein L4 pseudogene 4	9.25	SREBP-1(h),SREBP-2(h), MEF-2A(h), HLTF(h), POU2F1(h), MafK(h), Hox-A10(h), POU1F1(h)
ENSG00000216775		heterogeneous nuclear ribonucleoprotein A/B (HNRNPAB) pseudogene	9.25	SREBP-1(h),SREBP-2(h), PBX-1(h), HLTF(h), MEF-2A(h), Hox-A10(h), POU2F1(h), T3R-beta(h)...
ENSG00000204815	ODAD4	outer dynein arm docking complex subunit 4	9.21	Hox-A10(h), HLTF(h), POU1F1(h), FOXG1(h), PBX-1(h), MafK(h), POU2F1(h)...
ENSG00000162927	PUS10	pseudouridine synthase 10	9.06	HLTF(h), MEF-2A(h), POU1F1(h), MafK(h), POU2F1(h), Hox-A10(h)
ENSG00000153363	LINC00467	long intergenic non-protein coding RNA 467	9.01	MEF-2A(h), POU2F1(h), MafK(h), HLTF(h), POU1F1(h), FOXG1(h), Hox-A10(h)...

Enhancer model potentially involved in regulation of target genes (down-regulated genes in noRA_Dox vs. noRA_noDox).

To build the most specific composite modules we choose top 300 significant down-regulated genes as the input of CMA algorithm. The obtained CMA model is then applied to compute CMA score for all down-regulated genes in noRA_Dox vs. noRA_noDox.

The model consists of 2 module(s). Below, for each module the following information is shown:
- PWMs producing matches,
- number of individual matches for each PWM,
- score of the best match.

Module 1:

V$RELA_03	V$SP3_Q3	V$MAFG_Q3	V$TBX2_06	V$HNRPUL1_01	V$XBP1_09
0.94; N=2	0.83; N=2	0.72; N=2	0.98; N=3	0.99; N=2	0.94; N=2

Module width: 138

Module 2:

V$GCM1_08	V$GLI1_Q3_01	V$MAFG_06	V$CTF1_01
0.87; N=2	0.86; N=1	0.77; N=2	0.82; N=3

Module width: 114

Model score (-p*log10(pval)): 19.06
Wilcoxon p-value (pval): 9.22e-39
Penalty (p): 0.501
Average yes-set score: 8.50
Average no-set score: 6.77
AUC: 0.77
Separation point: 7.54
False-positive: 32.06%
False-negative: 23.57%

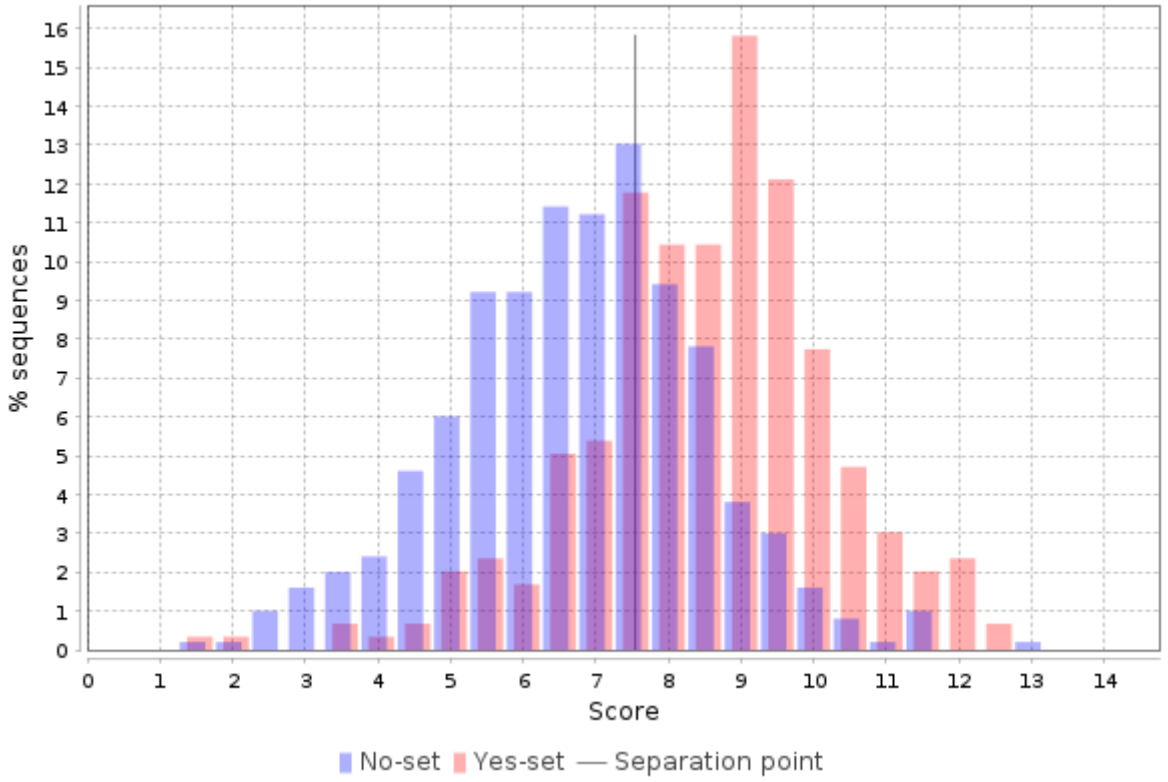


Table 5. List of top ten down-regulated genes in noRA_Dox vs. noRA_noDox with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000174791	RIN1	Ras and Rab interactor 1	13.7	GCMa(h), MafG(h), GLI1(h), NF-1C(h), TBX2(h), NF-kappaB-p65(h), HNRNPUL1(h)...
ENSG00000176978	DPP7	dipeptidyl peptidase 7	13.59	NF-1C(h), MafG(h), GCMa(h), GLI1(h), NF-kappaB-p65(h), XBP-1(h), TBX2(h)...
ENSG00000140320	BAHD1	bromo adjacent homology domain containing 1	13.41	NF-1C(h), MafG(h), GLI1(h), GCMa(h), TBX2(h), NF-kappaB-p65(h), HNRNPUL1(h)...
ENSG00000205903	ZNF316	zinc finger protein 316	13.33	MafG(h), TBX2(h), NF-kappaB-p65(h), Sp3(h), GLI1(h), GCMa(h), HNRNPUL1(h)
ENSG00000253982		novel transcript	13.31	TBX2(h), GCMa(h), GLI1(h), MafG(h), NF-kappaB-p65(h), Sp3(h), HNRNPUL1(h)
ENSG00000162430	SELENON	selenoprotein N	13.29	NF-1C(h), GLI1(h), GCMa(h), TBX2(h), NF-kappaB-p65(h), Sp3(h), HNRNPUL1(h)...
ENSG00000198835	GJC2	gap junction protein gamma 2	13.26	NF-1C(h), GCMa(h), Sp3(h), HNRNPUL1(h), NF-kappaB-p65(h), TBX2(h), GLI1(h)...
ENSG00000114554	PLXNA1	plexin A1	12.9	TBX2(h), NF-kappaB-p65(h), Sp3(h), HNRNPUL1(h), XBP-1(h), MafG(h), GCMa(h)...
ENSG00000136247	ZDHHC4	zinc finger DHHC-type palmitoyltransferase 4	12.89	MafG(h), TBX2(h), GCMa(h), GLI1(h), NF-1C(h), HNRNPUL1(h), NF-kappaB-p65(h)...
ENSG00000100092	SH3BP1	SH3 domain binding protein 1	12.88	MafG(h), GLI1(h), GCMa(h), NF-kappaB-p65(h), HNRNPUL1(h), NF-1C(h), Sp3(h)...

On the basis of the enhancer models we identified transcription factors potentially regulating the ***target genes*** of our interest. We found 11 and 9 transcription factors controlling expression of up- and down-regulated genes respectively (see Tables 6-7).

Table 6. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (up-regulated genes in noRA_Dox vs. noRA_noDox). ***Yes-No ratio*** *is the ratio between frequencies of the sites in Yes sequences versus No sequences. It describes the level of the enrichment of binding sites for the indicated TF in the regulatory target regions.* ***Regulatory score*** *is the measure of involvement of the given TF in the controlling of expression of genes that encode master regulators presented below (through positive feedback loops).*

See full table →

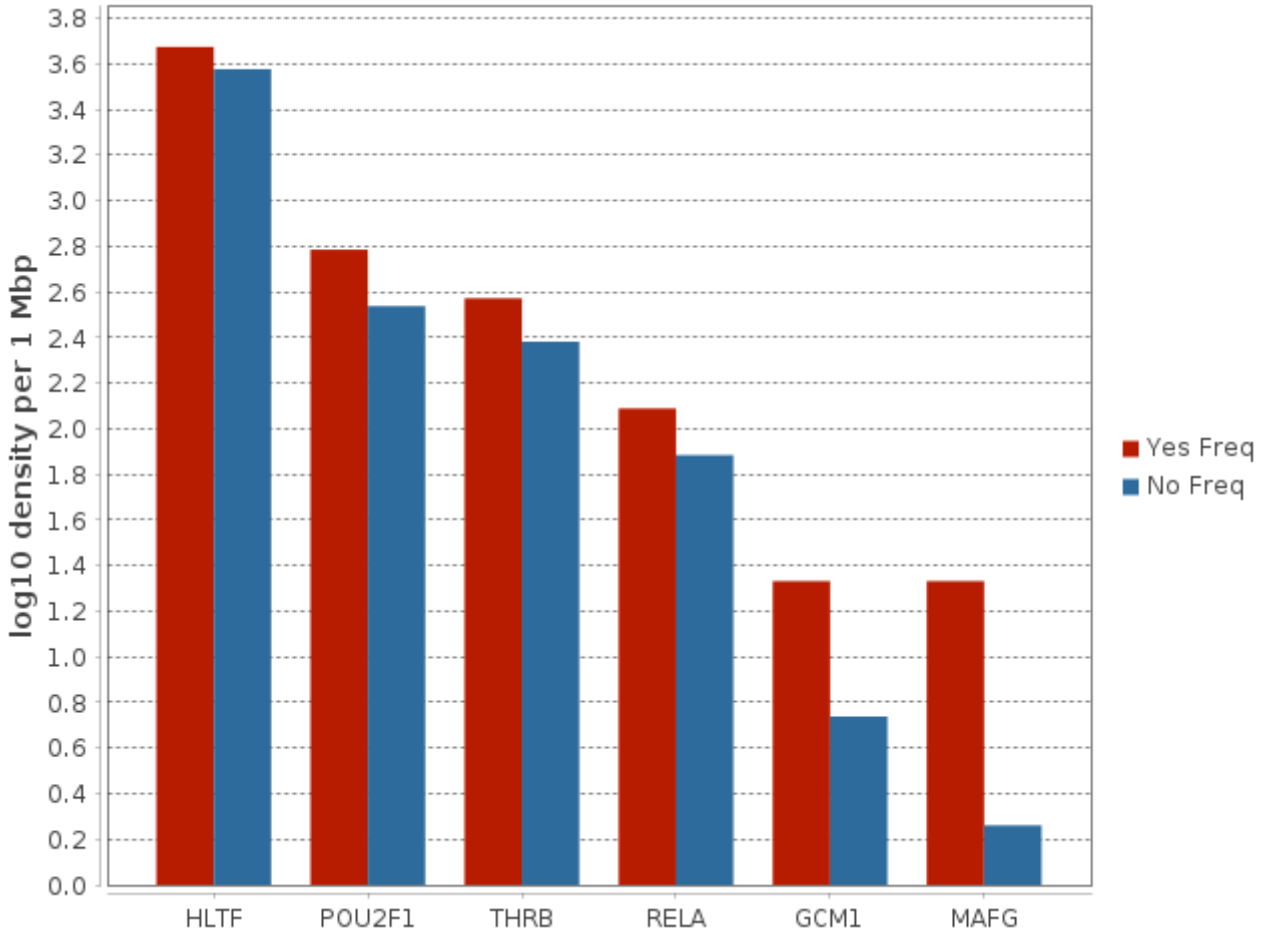
ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO000118284	HLTF	helicase like transcription factor	2.25	1.25
MO000025003	POU2F1	POU class 2 homeobox 1	2.2	1.77
MO000056537	THRB	thyroid hormone receptor beta	1.98	1.55
MO000028668	MAFK	MAF bZIP transcription factor K	1.9	1.73
MO000056029	SREBF1	sterol regulatory element binding transcription factor 1	1.89	2.16
MO000025765	SREBF2	sterol regulatory element binding transcription factor 2	1.89	2.66
MO000084966	MEF2A	myocyte enhancer factor 2A	1.78	1.9
MO000089495	HOXA10	homeobox A10	1.63	1.44
MO000084573	POU1F1	POU class 1 homeobox 1	1.52	1.45
MO000026342	FOXG1	forkhead box G1	0	2.34

Table 7. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (down-regulated genes in noRA_Dox vs. noRA_noDox). ***Yes-No ratio*** *is the ratio between frequencies of the sites in Yes sequences versus No sequences. It describes the level of the enrichment of binding sites for the indicated TF in the regulatory target regions.* ***Regulatory score*** *is the measure of involvement of the given TF in the controlling of expression of genes that encode master regulators presented below (through positive feedback loops).*

See full table →

ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO000079319	RELA	RELA proto-oncogene, NF-kB subunit	2.47	1.6
MO000026306	GCM1	glial cells missing transcription factor 1	1.67	3.92
MO000028667	MAFG	MAF bZIP transcription factor G	1.67	11.77
MO000019117	GLI1	GLI family zinc finger 1	1.66	1.54
MO000028209	TBX2	T-box transcription factor 2	1.62	1.47
MO000046079	SP3	Sp3 transcription factor	1.59	1.51
MO000061687	HNRNPUL1	heterogeneous nuclear ribonucleoprotein U like 1	1.36	1.34
MO000024750	NFIC	nuclear factor I C	1.08	11.21
MO000015029	XBP1	X-box binding protein 1	1	2.52

The following diagram represents the key transcription factors, which were predicted to be potentially regulating differentially expressed genes in the analyzed pathology: HLTF, POU2F1, THRB, RELA, GCM1 and MAFG.



3.4. Finding master regulators in networks

In the second step of the upstream analysis common regulators of the revealed TFs were identified. These master regulators appear to be the key candidates for therapeutic targets as they have a master effect on regulation of intracellular pathways that activate the pathological process of our study. The identified master regulators are shown in Tables 8-9.

*Table 8. Master regulators that may govern the regulation of **up-regulated** genes in noRA_Dox vs. noRA_noDox. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics data.*

See full table →

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO0000020219	Caspase-8(h)	CASP8	caspase 8	0.55	38
MO0000044264	SNCA(h)	SNCA	synuclein alpha	4.38	65
MO0000044265	SNCA-isoform1(h)	SNCA	synuclein alpha	4.38	65
MO0000103359	SNCA-isoform2(h)	SNCA	synuclein alpha	4.38	65
MO0000103362	SNCA-isoform3(h)	SNCA	synuclein alpha	4.38	65
MO0000480224	SNCA(h){pS129}	SNCA	synuclein alpha	4.38	65
MO0000044272	SNCA(h){gly}	SNCA	synuclein alpha	4.38	91
MO0000021036	Caspase-8(h)	CASP8	caspase 8	0.55	120
MO0000043060	(Caspase-8(h))2	CASP8	caspase 8	0.55	123
MO0000043221	Caspase-8a(h)	CASP8	caspase 8	0.55	166

*Table 9. Master regulators that may govern the regulation of **down-regulated** genes in noRA_Dox vs. noRA_noDox. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics data.*

See full table →

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO0000138295	ZBED1(h)	ZBED1	zinc finger BED-type containing 1	-0.56	32
MO0000124674	EPHB2(h)	EPHB2	EPH receptor B2	-0.77	96
MO0000124672	EPHB2-isoform1(h)	EPHB2	EPH receptor B2	-0.77	123
MO0000124673	EPHB2-isoform2(h)	EPHB2	EPH receptor B2	-0.77	123
MO0000255149	EPHB2-isoform3(h)	EPHB2	EPH receptor B2	-0.77	123
MO0000019174	Eck(h)	EPHA2	EPH receptor A2	-0.47	149
MO0000033313	PKACA(h)	PRKACA	protein kinase cAMP-activated catalytic subunit alpha	-0.43	198
MO0000334531	Eck-isoform2(h)	EPHA2	EPH receptor A2	-0.47	210
MO0000137320	Eck-isoform1(h)	EPHA2	EPH receptor A2	-0.47	211
MO0000032694	GPRK6(h)	GRK6	G protein-coupled receptor kinase 6	-0.44	247

The intracellular regulatory pathways controlled by the above-mentioned master regulators are depicted in Figures 9 and 10. These diagrams display the connections between identified transcription factors, which play important roles in the regulation of differentially expressed genes, and selected master regulators, which are responsible for the regulation of these TFs.

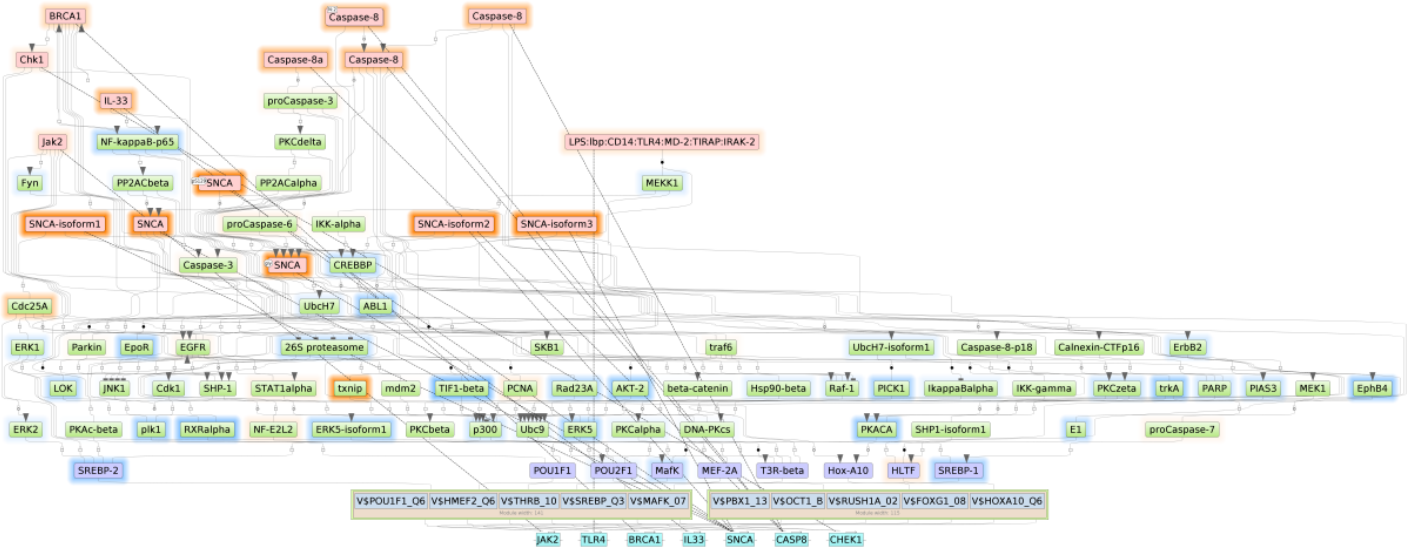


Figure 9. Diagram of intracellular regulatory signal transduction pathways of up-regulated genes in noRA_Dox vs. noRA_noDox. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp.

See full diagram →

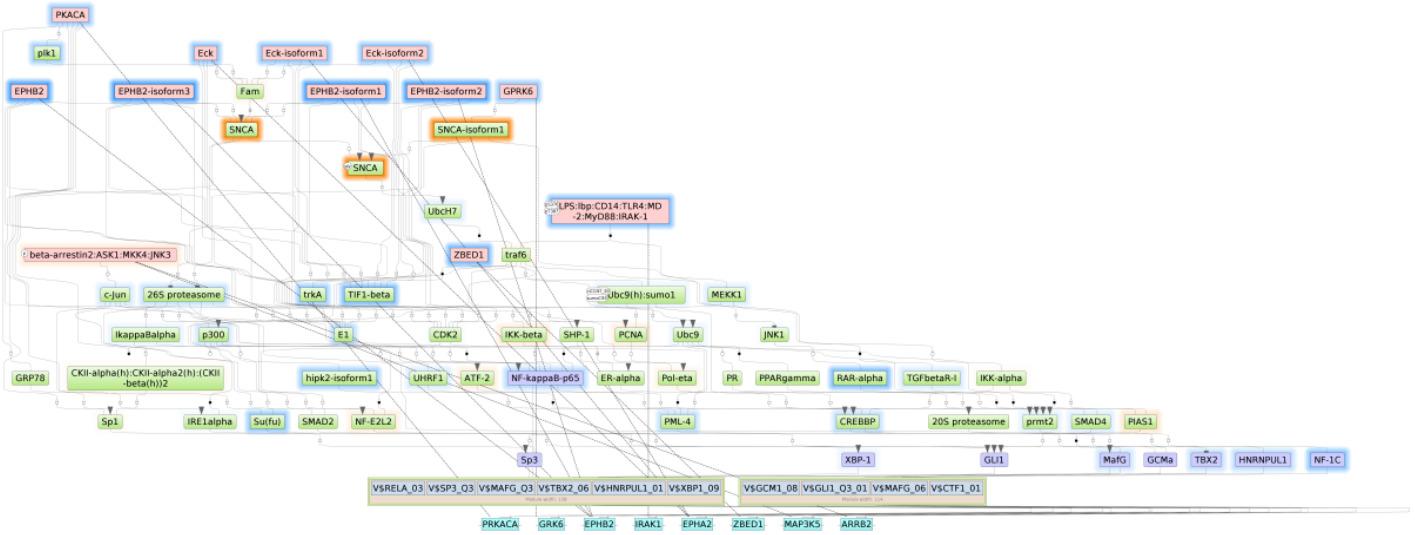


Figure 10. Diagram of intracellular regulatory signal transduction pathways of down-regulated genes in noRA_Dox vs. noRA_noDox. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp.

See full diagram →

4. Finding prospective drug targets

The identified master regulators that may govern pathology associated genes were checked for druggability potential using HumanPSD™ [5] database of gene-disease-drug assignments and PASS [11-13] software for prediction of biological activities of chemical compounds on the basis of a (Q)SAR approach. Respectively, for each master regulator protein we have computed two Druggability scores: HumanPSD Druggability score and PASS Druggability score. Where Druggability score represents the number of drugs that are potentially suitable for inhibition (or activation) of the corresponding target either according to the information extracted from medical literature (from HumanPSD™ database) or according to cheminformatics predictions of compounds activity against the examined target (from PASS software).

The cheminformatics druggability check is done using a pre-computed database of spectra of biological activities of chemical compounds from a library of all small molecular drugs from HumanPSD™ database, 2507 pharmaceutically active known chemical compounds in total. The spectra of biological activities has been computed using the program PASS [11-13] on the basis of a (Q)SAR approach.

If both Druggability scores were below defined thresholds (see Methods section for the details) such master regulator proteins were not used in further analysis of drug prediction.

As a result we created the following two tables of prospective drug targets (top targets are shown here):

*Table 10. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score from HumanPSD™ database. **Druggability score** contains the number of drugs that are potentially suitable for inhibition (or activation) of the target. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.*

See full table →

Gene symbol	Gene Description	Druggability score	logFC	Total rank
SNCA	synuclein alpha	1	4.38	91
PTPN2	protein tyrosine phosphatase non-receptor type 2	5	0.3	359
TEC	tec protein tyrosine kinase	30	0.51	373
CCNE2	cyclin E2	3	0.78	389
IRF1	interferon regulatory factor 1	1	0.5	448
S100B	S100 calcium binding protein B	4	0.4	448

*Table 11. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score predicted by PASS software. Here, the **Druggability score** for master regulator proteins is computed as a sum of PASS calculated probabilities to be active as a target for various small molecular compounds. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.*

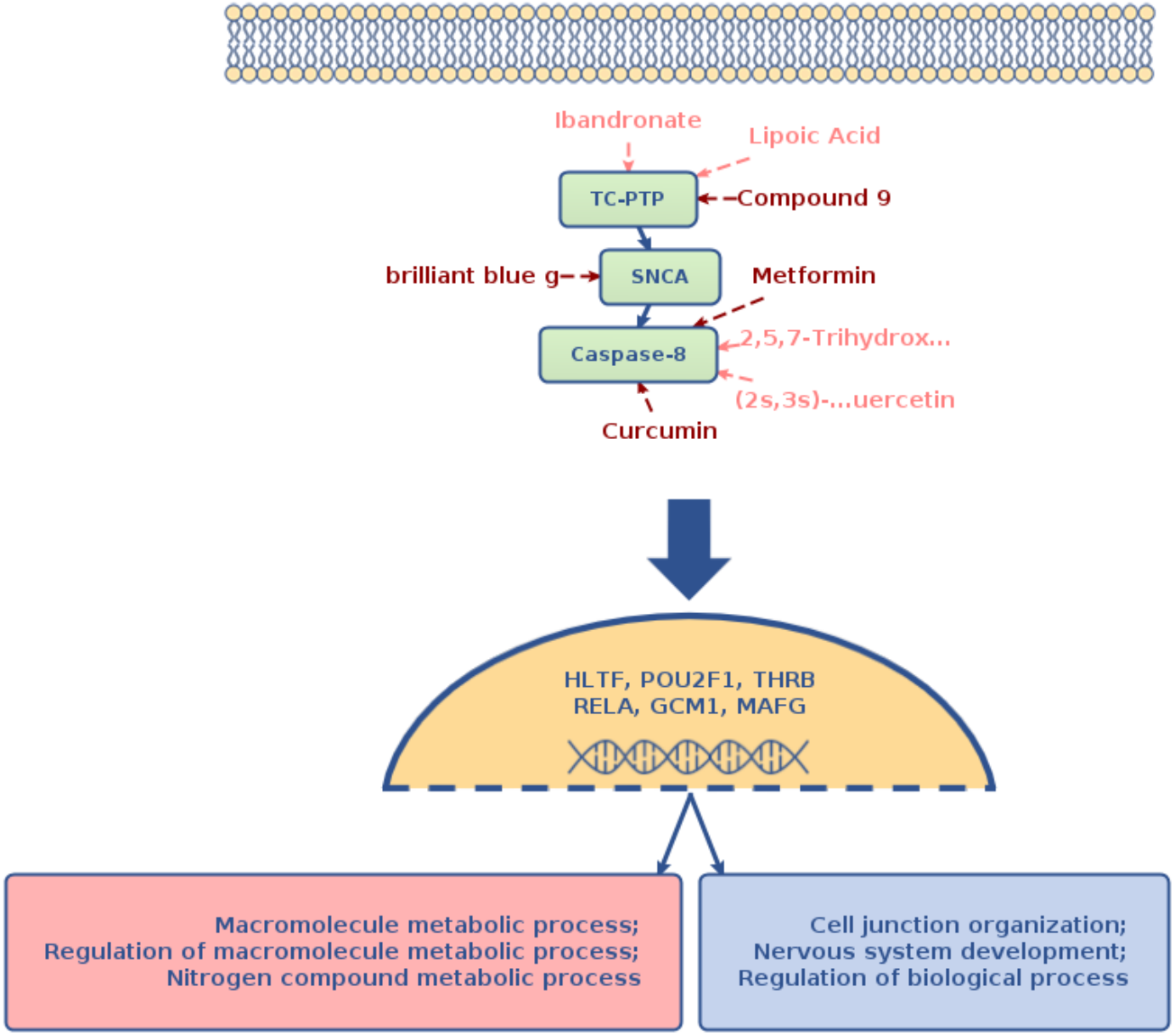
See full table →

Gene symbol	Gene Description	Druggability score	logFC	Total rank
PTPRK	protein tyrosine phosphatase receptor type K	17.53	0.19	315
PTPN2	protein tyrosine phosphatase non-receptor type 2	18.31	0.3	359
TEC	tec protein tyrosine kinase	7.34	0.51	373
LRRK2	leucine rich repeat kinase 2	3.25	0.32	375
CCNE2	cyclin E2	3.27	0.78	389
S100B	S100 calcium binding protein B	0	0.4	448

Below we represent schematically the main mechanism of the studied pathology. In the schema we considered the top two drug targets of each of the two categories computed above. In addition we have added two top identified master regulators for which no drugs may be identified yet, but that are playing the crucial role in the molecular mechanism of the studied pathology. Thus the molecular mechanism of the studied pathology was predicted to be mainly based on the following key master regulators:

- Caspase-8
- TC-PTP
- SNCA

This result allows us to suggest the following schema of affecting the molecular mechanism of the studied pathology:



Drugs which are shown on this schema: Lipoic Acid, 2,5,7-Trihydroxynaphthoquinone, brilliant blue g, (2s,3s)-Trans-Dihydroquercetin, Ibandronate, Compound 9, Curcumin and Metformin, should be considered as a prospective research initiative for further drug repurposing and drug development. These drugs were selected as top matching treatments to the most prospective drug targets of the studied pathology, however, these results should be considered with special caution and are to be used for research purposes only, as there is not enough clinical information for adapting these results towards immediate treatment of patients.
The drugs given in dark red color on the schema are FDA approved drugs or drugs which have gone through various phases of clinical trials as active treatments against the selected targets.
The drugs given in pink color on the schema are drugs, which were cheminformatically predicted to be active against the selected targets.

5. Identification of potential drugs

In the last step of the analysis we strived to identify known activities as well as drugs with cheminformatically predicted activities that are potentially suitable for inhibition (or activation) of the identified molecular targets in the context of specified human diseases(s).

Proposed drugs are top ranked drug candidates, that were found to be active on the identified targets and were selected from 4 categories:

1. FDA approved drugs or used in clinical trials drugs for the studied pathology;
2. Repurposing drugs used in clinical trials for other pathologies;

3. Drugs, predicted by PASS to be active against identified drug targets and against the studied pathology;
4. Drugs, predicted by PASS to be active against identified drug targets but for other pathologies.

Proposed drugs were selected on the basis of Drug rank which was computed from the ranks sum based on the individual ranks of the following scores:

- Target activity score (depends on ranks of all targets that were found for the selected drug);
- Disease activity score (weighted sum of number of clinical trials on disease(s) under study where the selected drug is known to be applied or PASS Disease activity score - cheminformatically predicted property of the compound to be active against the studied disease(s));
- Clinical validity score (applicable only for drugs predicted on the basis of literature curation in HumanPSD™ database (Tables 12 and 13), reflects the number of the highest clinical trials phase on which the drug was tested for any pathology).

You can refer to the Methods section for more details on drug ranking procedure.

Based on the Drug rank, a numerical value of Drug score was calculated, which reflects the potential activity of the respective drug on the overall molecular mechanism of the studied pathology. Drug score values belong to the range from 1 to 100 and are calculated as a quotient of maximum drug rank and the drug rank of the given drug multiplied by 100.

Top drugs of each category are given in the tables below:

Drugs approved in clinical trials

Table 12. FDA approved drugs or drugs used in clinical trials for the studied pathology (most promising treatment candidates selected for the identified drug targets on the basis of literature curation in HumanPSD™ database)
See full table →

Name	Target names	Drug score	Disease activity score	Disease trial phase
Sirolimus	IKBKB, MAPK10, RPS6KA3, ROCK2, DYRK1A, FGF2, HSP90AA1, TGM2, NFE2L2, MAPK12, CHEK1, PRKD1, RPS6KB1	90	3	Phase 2: Parkinson Disease, Acute Disease, Adenocarcinoma, Adenocarcinoma, Mucinous, Adenoma, Adenoma, Islet Cell, Adenomatous Polyposis Coli, Adenomatous Polyps, Alzheimer Disease, Amyotrophic Lateral Sclerosis, Anemia, Anemia, Aplastic, Anemia, Hemolytic, Anemia, Hemolytic, Autoimmune, Anemia, Refractory, Anemia, Refractory, with Excess of Blasts, Anemia, Sickle Cell, Angina Pectoris, Angina, Unstable, Angiofibroma, Angiomyolipoma, Angiomyoma, Aphasia, Aphasia, Primary Progressive, Arteriovenous Malformations, Atrophy, Autoimmune Lymphoproliferative Syndrome, Blast Crisis, Bone Marrow Failure Disorders, Brain Abscess, Brain Stem Infarctions, Breast Neoplasms, Bronchiolitis, Bronchiolitis Obliterans, Bronchiolitis Obliterans Syndrome, Burkitt Lymphoma, COVID-19, Carcinoma, Carcinoma, Endometrioid, Carcinoma, Hepatocellular, Carcinoma, Non-Small-Cell Lung, Carcinoma, Renal Cell, Carcinoma, Squamous Cell, Chordoma, Cockayne Syndrome, Cognitive Dysfunction, Colorectal Neoplasms, Congenital Abnormalities, Coronary Artery Disease, Coronary Disease, Cysts, Cytopenia, Dementia, Depression, Depressive Disorder, Diabetes Mellitus, Diabetes Mellitus, Type 1, Diabetic Retinopathy, Dilatation, Pathologic, Edema, Endometrial Neoplasms, Epidermolysis Bullosa, Epidermolysis Bullosa Simplex, Epilepsy, Epistaxis, Erythema, Esophageal Squamous Cell Carcinoma, Eye Diseases, Fanconi Anemia, Fanconi Syndrome, Fibroma, Fibrosarcoma, Fibrosis, Frontotemporal Dementia, Ganglion Cysts, Ganglioneuroblastoma, Genital Diseases, Genital Diseases, Female, Geographic Atrophy, Giant Lymph Node Hyperplasia, Glioblastoma, Glioma, Gliosarcoma, Glomerulonephritis, Glomerulonephritis, IGA, Gout, Graft vs Host Disease, Graves Ophthalmopathy, Hamartoma, Hamartoma Syndrome, Multiple, Head and Neck Neoplasms, Hemangioendothelioma, Hemangioma, Hemangiosarcoma, Hematologic Diseases, Hematologic Neoplasms, Hemolysis, Hodgkin Disease, Hyperinsulinism, Hypertension, Hyperuricemia, Hypoglycemia, Immunologic Deficiency Syndromes, Infarction, Inflammatory Bowel Diseases, Intestinal Neoplasms, Intracranial Arteriovenous Malformations, Ischemia, Kasabach-Merritt Syndrome, Kidney Diseases, Kidney Diseases, Cystic, Kidney Failure, Chronic, Klippel-Trenaunay-Weber Syndrome, Leiomyoma, Leiomyomatosis, Leiomyosarcoma, Leukemia, Leukemia, Biphenotypic, Acute, Leukemia, Lymphocytic, Chronic, B-Cell, Leukemia, Lymphoid, Leukemia, Myelogenous, Chronic, BCR-ABL Positive, Leukemia, Myeloid, Leukemia, Myeloid, Accelerated Phase, Leukemia, Myeloid, Acute, Leukemia, Myeloid, Chronic, Atypical, BCR-ABL Negative, Leukemia, Myeloid, Chronic-Phase, Leukemia, Myelomonocytic, Chronic, Leukemia, Myelomonocytic, Juvenile, Leukemia, Plasma Cell, Leukemia, Prolymphocytic, Leukemia, Prolymphocytic, T-Cell, Leukemia, Promyelocytic, Acute, Lipoma, Liposarcoma, Liver Neoplasms, Lung Neoplasms, Lupus Erythematosus, Systemic, Lupus Nephritis, Lymphangioleiomyomatosis, Lymphangioma, Lymphangiomyoma, Lymphatic Abnormalities, Lymphedema, Lymphoma, Lymphoma, B-Cell, Lymphoma, B-Cell, Marginal Zone, Lymphoma, Follicular, Lymphoma, Large B-Cell, Diffuse, Lymphoma, Large-Cell, Anaplastic, Lymphoma, Large-Cell, Immunoblastic, Lymphoma, Mantle-Cell, Lymphoma, Non-Hodgkin, Lymphoma, T-Cell, Lymphoproliferative Disorders, Macular Degeneration, Macular Edema, Melanoma, Metabolic Diseases, Motor Neuron Disease, Mouth Neoplasms, Multiple Myeloma, Multiple System Atrophy, Myelodysplastic Syndromes, Myelodysplastic-Myeloproliferative Diseases, Myeloproliferative Disorders, Myocardial Infarction, Myocardial

				Ischemia, Myoma, Myosarcoma, Myxoma, Nasopharyngeal Carcinoma, Nasopharyngeal Neoplasms, Neoplasm Metastasis, Neoplasm, Residual, Neoplasms, Neoplasms, Plasma Cell, Neoplasms, Second Primary, Nephritis, Nerve Sheath Neoplasms, Neurilemmoma, Neuroblastoma, Neuroectodermal Tumors, Neuroectodermal Tumors, Primitive, Neuroectodermal Tumors, Primitive, Peripheral, Neuroendocrine Tumors, Neurofibroma, Neurofibromatoses, Neurofibromatosis 1, Neurofibrosarcoma, Neutropenia, Osteosarcoma, Ovarian Diseases, Ovarian Neoplasms, Pancreatic Neoplasms, Pancytopenia, Panuveitis, Pars Planitis, Peripheral Arterial Disease, Peripheral Vascular Diseases, Peritoneal Fibrosis, Peritoneal Neoplasms, Pharyngeal Neoplasms, Pica, Pick Disease of the Brain, Plasmablastic Lymphoma, Plasmacytoma, Pneumonia, Polycystic Kidney Diseases, Polycystic Kidney, Autosomal Dominant, Polyps, Post-Acute COVID-19 Syndrome, Precancerous Conditions, Precursor Cell Lymphoblastic Leukemia-Lymphoma, Preleukemia, Primary Myelofibrosis, Prostatic Neoplasms, Pseudomyxoma Peritonei, Pulmonary Fibrosis, Purpura, Purpura, Thrombocytopenic, Purpura, Thrombocytopenic, Idiopathic, Rage, Rectal Neoplasms, Recurrence, Renal Insufficiency, Renal Insufficiency, Chronic, Retinal Diseases, Retroperitoneal Fibrosis, Retroperitoneal Neoplasms, Rhabdomyosarcoma, Rhabdomyosarcoma, Embryonal, ST Elevation Myocardial Infarction, Sarcoidosis, Sarcoma, Sarcoma, Alveolar Soft Part, Sarcoma, Ewing, Sarcoma, Kaposi, Sarcoma, Synovial, Scleritis, Sclerosis, Severe Acute Respiratory Syndrome, Severe Combined Immunodeficiency, Shy-Drager Syndrome, Squamous Cell Carcinoma of Head and Neck, Stomach Neoplasms, Sturge-Weber Syndrome, Syndrome, Telangiectasia, Hereditary Hemorrhagic, Telangiectasis, Thalassemia, Thrombosis, Tongue Neoplasms, Triple Negative Breast Neoplasms, Tuberous Sclerosis, Uveitis, Uveitis, Intermediate, Uveitis, Posterior, Vascular Diseases, Vascular Malformations, Vitiligo, Waldenstrom Macroglobulinemia, Wet Macular Degeneration, alpha-Thalassemia, beta-Thalassemia
Moxifloxacin	TOP2B	64	1	Phase 1: Parkinson Disease, Acne Vulgaris, Acute Coronary Syndrome, Alzheimer Disease, Amyotrophic Lateral Sclerosis, Anemia, Autoimmune Diseases, Bacterial Infections, Carcinoma, Renal Cell, Cardiovascular Diseases, Cataract, Constipation, Coronary Artery Disease, Coronary Disease, Cystic Fibrosis, Cysts, Depressive Disorder, Depressive Disorder, Major, Diabetes Mellitus, Diabetes Mellitus, Type 2, Dyslipidemias, Endometriosis, Endophthalmitis, Epilepsy, Erectile Dysfunction, Fibromyalgia, HIV Infections, Heart Failure, Hemorrhage, Hepatitis, Hepatitis C, Hot Flashes, Hyperlipidemias, Hypertension, Hyperuricemia, Hypoglycemia, Immune System Diseases, Infections, Influenza, Human, Ischemia, Lipid Metabolism Disorders, Lung Diseases, Malaria, Malaria, Falciparum, Malaria, Vivax, Migraine Disorders, Motor Neuron Disease, Myelitis, Myocardial Ischemia, Obesity, Osteomyelitis, Postpartum Hemorrhage, Psychotic Disorders, Pulmonary Arterial Hypertension, Pulmonary Disease, Chronic Obstructive, Restless Legs Syndrome, Schizophrenia, Tic Disorders, Tuberculosis, Tuberculosis, Multidrug-Resistant, Urinary Bladder, Overactive, Vascular Diseases
Capsaicin	NFE2L2, PCNA	56	4	Phase 2: Parkinson Disease, Arthritis, Cough, Cystitis, Cystitis, Interstitial, Cysts, Diabetes Mellitus, Diabetic Neuropathies, HIV Infections, Herpes Zoster, Hyperemesis Gravidarum, Hypertension, Hypertension, Pulmonary, Infections, Ischemic Stroke, Knee Injuries, Nausea, Nervous System Diseases, Neuralgia, Neuroma, Osteoarthritis, Osteoarthritis, Knee, Pain, Peripheral Nervous System Diseases, Polyneuropathies, Pregnancy Complications, Pruritus, Pulmonary Arterial Hypertension, Stroke, Syncope, Syncope, Vasovagal, Tennis Elbow, Vomiting, Vulvar Diseases, Vulvar Vestibulitis, Wounds and Injuries
L-Sulforaphane	MAPK10, NFE2L2, MAPK12, CD44	56	2	Phase 2: Parkinson Disease, Autism Spectrum Disorder, Autistic Disorder, Child Development Disorders, Pervasive, Depression, Depressive Disorder, Developmental Disabilities, Mental Disorders, Neurodevelopmental Disorders, Psychotic Disorders, Schizophrenia, Tic Disorders
Metformin	CASP8	48	2	Phase 2: Parkinson Disease, Acne Vulgaris, Acute Kidney Injury, Adenocarcinoma, Adenocarcinoma of Lung, Alzheimer Disease, Amyotrophic Lateral Sclerosis, Anemia, Aphasia, Aphasia, Primary

Progressive, Apnea, Arthritis, Arthritis, Rheumatoid, Arthrogryposis, Ascites, Asthma, Atrial Fibrillation, Atrophy, Bardet-Biedl Syndrome, Barrett Esophagus, Behavior, Blindness, Brain Abscess, Brain Neoplasms, Breast Neoplasms, Brenner Tumor, COVID-19, Carcinoma, Carcinoma, Adenosquamous, Carcinoma, Endometrioid, Carcinoma, Non-Small-Cell Lung, Carcinoma, Ovarian Epithelial, Carcinoma, Small Cell, Carcinoma, Squamous Cell, Cardiovascular Diseases, Cerebral Palsy, Cholangiocarcinoma, Cholestasis, Cholestasis, Intrahepatic, Chondrosarcoma, Chromosome Disorders, Chronic Periodontitis, Coinfection, Colitis, Colitis, Ulcerative, Colorectal Neoplasms, Communicable Diseases, Coronary Artery Disease, Coronary Disease, Cystadenocarcinoma, Cystadenocarcinoma, Serous, Cystadenoma, Serous, Cystic Fibrosis, Cysts, Cytopenia, Dementia, Dementia, Vascular, Dengue, Diabetes Mellitus, Diabetes Mellitus, Type 2, Diabetes, Gestational, Diabetic Foot, Disease Progression, Dyslipidemias, Endocrine System Diseases, Erythroplasia, Esophageal Neoplasms, Esophageal Squamous Cell Carcinoma, Fallopian Tube Neoplasms, Familial Primary Pulmonary Hypertension, Fanconi Anemia, Fanconi Syndrome, Fatty Liver, Fatty Liver, Alcoholic, Fibromyalgia, Fibrosis, Fragile X Syndrome, Frailty, Frontotemporal Dementia, Furcation Defects, Genetic Diseases, Inborn, Genetic Diseases, X-Linked, Geographic Atrophy, Germinoma, Glioblastoma, Glioma, Glucose Intolerance, Glucose Metabolism Disorders, HIV Infections, HIV Seropositivity, Head and Neck Neoplasms, Hearing Loss, Hearing Loss, Sensorineural, Heart Failure, Heart Failure, Systolic, Hemangiosarcoma, Hepatitis, Hepatitis A, Hepatitis C, Hepatitis C, Chronic, Hepatitis, Chronic, Hyperandrogenism, Hypercholesterolemia, Hyperglycemia, Hyperinsulinism, Hyperlactatemia, Hyperlipidemias, Hyperplasia, Hypersensitivity, Hypertension, Hypertension, Pulmonary, Infarction, Infections, Infertility, Infertility, Female, Inflammation, Inflammatory Bowel Diseases, Insulin Resistance, Intellectual Disability, Iron Metabolism Disorders, Ischemia, Kidney Diseases, Kidney Diseases, Cystic, Laurence-Moon Syndrome, Leprosy, Leprosy, Multibacillary, Leukemia, Leukoplakia, Leukoplakia, Oral, Liver Diseases, Low Back Pain, Lung Neoplasms, Lymphoma, Lymphoma, Follicular, Lymphoma, Large B-Cell, Diffuse, Macular Degeneration, Melanoma, Menstruation Disturbances, Mental Retardation, X-Linked, Metabolic Diseases, Microvascular Angina, Mitochondrial Diseases, Monoclonal Gammopathy of Undetermined Significance, Motor Neuron Disease, Mouth Neoplasms, Movement Disorders, Multiple Myeloma, Multiple Sclerosis, Multiple Sclerosis, Chronic Progressive, Multiple Sclerosis, Relapsing-Remitting, Myelodysplastic Syndromes, Myocardial Infarction, Myocardial Ischemia, Myofascial Pain Syndromes, Neoplasm Metastasis, Neoplasms, Neoplasms, Germ Cell and Embryonal, Neoplasms, Plasma Cell, Nerve Degeneration, Neurobehavioral Manifestations, Non-alcoholic Fatty Liver Disease, Obesity, Obesity, Morbid, Obstetric Labor, Premature, Optic Atrophy, Oral Manifestations, Osteoarthritis, Osteoarthritis, Knee, Osteosarcoma, Ovarian Neoplasms, Paralysis, Paraproteinemias, Peptic Ulcer, Periodontitis, Peritoneal Neoplasms, Pick Disease of the Brain, Pleural Effusion, Pleural Effusion, Malignant, Polycystic Kidney Diseases, Polycystic Kidney, Autosomal Dominant, Polycystic Ovary Syndrome, Prediabetic State, Pregnancy Complications, Preleukemia, Premature Birth, Prostatic Neoplasms, Pulmonary Arterial Hypertension, Rectal Neoplasms, Recurrence, Renal Insufficiency, Renal Insufficiency, Chronic, Retinal Degeneration, Retinal Diseases, Retinal Dystrophies, ST Elevation Myocardial Infarction, Sarcoidosis, Sarcoidosis, Pulmonary, Sarcoma, Sarcoma, Ewing, Sclerosis, Sepsis, Severe Acute Respiratory Syndrome, Sex Chromosome Disorders, Sex Chromosome Disorders of Sex Development, Shock, Shock, Septic, Sleep Apnea Syndromes, Sleep Apnea, Obstructive, Small Cell Lung Carcinoma, Smoldering Multiple Myeloma, Stargardt Disease, Stroke, Syndrome, Toxemia, Triple Negative Breast Neoplasms, Tuberculosis, Tuberculosis, Pulmonary, Ulcer, Uterine Cervical Neoplasms, Vascular Diseases, Virus Diseases, Vision Disorders, Vision, Low, Vitiligo, Weight Loss, Wolfram Syndrome, Wounds and Injuries, Yellow Fever

The ***Disease trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for the analyzed pathology.

Repurposing drugs

Table 13. Repurposed drugs used in clinical trials for other pathologies (prospective drugs against the identified drug targets on the basis of literature curation in HumanPSD™ database)
See full table →

Name	Target names	Drug score	Maximum trial phase
1-(5-Tert-Butyl-2-P-Tolyl-2h-Pyrazol-3-Yl)-3-[4-(2-Morpholin-4-Yl-Ethoxy)-Naphthalen-1-Yl]-Urea	IKBKB, MAPK10, RPS6KA3, TEC, ROCK2, MAP4K4, PRKCQ, TTK, LATS1, SLK, MAP2K4, CDK7, ACVR2A, EIF2AK2, PRKG1, CHEK1, JAK2, MAP3K20, PRKD1, RPS6KB1, RIPK2, TNIK, MERTK, LATS2, DYRK1A, NUAK1, CLK4, MAPK12, WEE1, EPHA6, TGFBR2, CLK1, PLK4	95	Phase 2: Arthritis, Arthritis, Rheumatoid, Psoriasis
pi-103	IKBKB, MAPK10, RPS6KA3, TEC, ROCK2, MAP4K4, PRKCQ, TTK, LATS1, SLK, MAP2K4, CDK7, ACVR2A, EIF2AK2, PRKG1, CHEK1, JAK2, MAP3K20, PRKD1, RPS6KB1, RIPK2, TNIK, MERTK, LATS2, DYRK1A, NUAK1, CLK4, MAPK12, WEE1, EPHA6, TGFBR2, CLK1, PLK4	95	N/A
seliciclib	IKBKB, MAPK10, RPS6KA3, TEC, ROCK2, MAP4K4, PRKCQ, TTK, LATS1, SLK, MAP2K4, CDK7, ACVR2A, EIF2AK2, PRKG1, CHEK1, JAK2, MAP3K20, PRKD1, RPS6KB1, RIPK2, TNIK, MERTK, LATS2, DYRK1A, NUAK1, CLK4, MAPK12, WEE1, EPHA6, TGFBR2, CLK1, PLK4	95	Phase 2: Cystic Fibrosis, Cysts, Fibrosis
ruboxistaurin	IKBKB, MAPK10, RPS6KA3, TEC, ROCK2, MAP4K4, PRKCQ, TTK, LATS1, SLK, MAP2K4, CDK7, ACVR2A, EIF2AK2, PRKG1, CHEK1, JAK2, MAP3K20, PRKD1, RPS6KB1, RIPK2, TNIK, MERTK, LATS2, DYRK1A, NUAK1, CLK4, MAPK12, WEE1, EPHA6, TGFBR2, CLK1, PLK4	95	Phase 3: Diabetes Mellitus, Diabetes Mellitus, Type 1, Diabetes Mellitus, Type 2, Diabetic Neuropathies, Diabetic Retinopathy, Edema, Macular Edema, Nervous System Diseases, Peripheral Nervous System Diseases, Retinal Diseases
Sorafenib	IKBKB, MAPK10, RPS6KA3, TEC, ROCK2, MAP4K4, PRKCQ, TTK, LATS1, SLK, MAP2K4, CDK7, ACVR2A, EIF2AK2, PRKG1, CHEK1, JAK2, MAP3K20, PRKD1, RPS6KB1, RIPK2, TNIK, MERTK, LATS2, DYRK1A, NUAK1, CLK4, MAPK12, WEE1, EPHA6, TGFBR2, CLK1, PLK4	95	Phase 4: Carcinoma, Carcinoma, Hepatocellular, Carcinoma, Renal Cell, Thrombosis

The ***Maximum trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for any pathology.

Table 14. Prospective drugs, predicted by PASS software to be active against the identified drug targets with predicted activity against the studied disease(s) (drug candidates predicted with the cheminformatics tool PASS)
See full table →

Name	Target names	Drug score	Target activity score
Lipoic Acid	CDC25A, PTPRD, PTPRO, PTPRJ, EPM2A, PTPN2, PPM1D, PTPN13, PTPN12, CDC25C, PTPRK, UBASH3B	94	0.44
Tiludronate	CDC25A, PTPRD, PTPRO, PTPRJ, EPM2A, PTPN2, PPM1D, IL33, PTPN13, PTPN12, CDC25C, PTPRK, UBASH3B	92	0.32
[[N-(Benzyloxycarbonyl)Amino]Methyl]Phosphate	CDC25A, PTPRD, PTPRO, PTPRJ, EPM2A, PTPN2, PPM1D, PTPN13, PTPN12, CDC25C, PTPRK, UBASH3B	90	0.31
3-(Phosphonomethyl)Pyridine-2-Carboxylic Acid	CDC25A, PTPRD, PTPRO, PTPRJ, EPM2A, PTPN2, PPM1D, PTPN13, PTPN12, CDC25C, PTPRK, UBASH3B	89	0.35
Tacrolimus	PPP3CA, PPM1D, IL33	88	7.3E-2

Table 15. Prospective drugs, predicted by PASS software to be active against the identified drug targets, though without cheminformatically predicted activity against the studied disease(s) (drug candidates predicted with the cheminformatics tool PASS)
See full table →

Name	Target names	Drug score	Target activity score
2,5,7-Trihydroxynaphthoquinone	MAPK10, CDC25A, MAPK12, SENP6, EPM2A, CASP8, DYRK1A, SENP8, BRCA1, HSP90AA1	83	0.36
Isoformononetin	MAPK10, NFE2L2, MAPK12, CASP8, HIF1A, BRCA1, HSP90AA1	82	0.16
Bortezomib	PLAT, PSMA3, PSMA4, PSMD5	82	0.13
N-(4-MORPHOLINE)CARBONYL-B-(1-NAPHTHYL)-L-ALANINE-L-LEUCINE BORONIC ACID	PLAT, PSMA3, PSMA4, PSMD5	81	0.12
Thioproline	ITGA6, IL33, ITGB1, ITGAV, ITGA1	81	0.16

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: Sirolimus, 1-(5-Tert-Butyl-2-P-Tolyl-2h-Pyrazol-3-Yl)-3-[4-(2-Morpholin-4-Yl-Ethoxy)-Naphthalen-1-Yl]-Urea, Lipoic Acid and 2,5,7-Trihydroxynaphthoquinone. These drugs were selected for acting on the following targets: PRKD1, TEC, PTPRK and CASP8, which were predicted to be active in the molecular mechanism of the studied pathology.

The selected drugs are top ranked drug candidates from each of the four categories of drugs: (1) FDA approved drugs or used in clinical trials drugs for the studied pathology; (2) repurposing drugs used in clinical trials for other pathologies; (3) drugs, predicted by PASS software to be active against the studied pathology; (4) drugs, predicted by PASS software to be repurposed from other pathologies.

6. Conclusion

We applied the software package "Genome Enhancer" to a data set that contains *transcriptomics* data. The study is done in the context of *Parkinson Disease*. The data were pre-processed, statistically analyzed and differentially expressed genes were identified. Also checked was the enrichment of GO or disease categories among the studied gene sets.

We propose the following drugs as most promising candidates for treating the pathology under study:

Sirolimus, 1-(5-Tert-Butyl-2-P-Tolyl-2h-Pyrazol-3-Yl)-3-[4-(2-Morpholin-4-Yl-Ethoxy)-Naphthalen-1-Yl]-Urea, Lipoic Acid and 2,5,7-Trihydroxynaphthoquinone

These drugs were selected for acting on the following targets: PRKD1, TEC, PTPRK and CASP8, which were predicted to be involved in the molecular mechanism of the pathology under study.

The identified molecular mechanism of the studied pathology was predicted to be mainly based on the following key drug targets:

Caspase-8, TC-PTP and SNCA

These potential drug targets should be considered as a prospective research initiative for further drug repurposing and drug development purposes. The following drugs were predicted as, matching those drug targets: Lipoic Acid, 2,5,7-Trihydroxynaphthoquinone, brilliant blue g, (2s,3s)-Trans-Dihydroquercetin, Ibandronate, Compound 9, Curcumin and Metformin. These drugs should be considered with special caution for research purposes only.

In this study, we came up with a detailed signal transduction network regulating differentially expressed genes in the studied pathology. In this network we have revealed the following top master regulators (signaling proteins and their complexes) that play a crucial role in the molecular mechanism of the studied pathology, which can be proposed as the most promising molecular targets for further drug repurposing and drug development initiatives.

- Caspase-8
- TC-PTP
- SNCA

Potential drug compounds which can be affecting these targets can be found in the "Finding prospective drug targets" section.

7. Methods

Databases used in the study

Transcription factor binding sites in promoters and enhancers of differentially expressed genes were analyzed using known DNA-binding motifs described in the TRANSFAC® library, release 2024.1 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transfac).
The master regulator search uses the TRANSPATH® database (BIOBASE), release 2024.1 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transpath). A comprehensive signal transduction network of human cells is built by the software on the basis of reactions annotated in TRANSPATH®.
The information about drugs corresponding to identified drug targets and clinical trials references were extracted from HumanPSD™ database, release 2024.1 (https://genexplain.com/humanpsd).
The Ensembl database release Human104.38 (hg38) (http://www.ensembl.org) was used for gene IDs representation and Gene Ontology (GO) (http://geneontology.org) was used for functional classification of the studied gene set.

Methods for the analysis of enriched transcription factor binding sites and composite modules

Transcription factor binding sites in promoters and enhancers of differentially expressed genes were analyzed using known DNA-binding motifs. The motifs are specified using position weight matrices (PWMs) that give weights to each nucleotide in each position of the DNA binding motif for a transcription factor or a group of them.

We search for transcription factor binding sites (TFBS) that are enriched in the promoters and enhancers under study as compared to a background sequence set such as promoters of genes that were not differentially regulated under the condition of the experiment. We denote study and background sets briefly as Yes and No sets. In the current work we used a workflow considering promoter sequences of a standard length of 1100 bp (-1000 to +100). The error rate in this part of the pipeline is controlled by estimating the adjusted p-value (using the Benjamini-Hochberg procedure) in comparison to the TFBS frequency found in randomly selected regions of the human genome (adj.p-value < 0.01).
We have applied the CMA algorithm (Composite Module Analyst) for searching composite modules [7] in the promoters and enhancers of the Yes and No sets. We searched for a composite module consisting of a cluster of 10 TFs in a sliding window of 200-300 bp that statistically significantly separates sequences in the Yes and No sets (minimizing Wilcoxon p-value).

Methods for finding master regulators in networks

We searched for master regulator molecules in signal transduction pathways upstream of the identified transcription factors. The master regulator search uses a comprehensive signal transduction network of human cells. The main algorithm of the master regulator search has been described earlier [3,4]. The goal of the algorithm is to find nodes in the global signal transduction network that may potentially regulate the activity of a set of transcription factors found at the previous step of the analysis. Such nodes are considered as most promising drug targets, since any influence on such a node may switch the transcriptional programs of hundreds of genes that are regulated by the respective TFs. In our analysis, we have run the algorithm with a maximum radius of 12 steps upstream of each TF in the input set. The error rate of this algorithm is controlled by applying it 10000 times to randomly generated sets of input transcription factors of the same set-size. Z-score and FDR value of ranks are calculated then for each potential master regulator node on the basis of such random runs (see detailed description in [9]). We control the error rate by the FDR threshold 0.05.

Methods for analysis of pharmaceutical compounds

We seek for the optimal combination of molecular targets (key elements of the regulatory network of the cell) that potentially interact with pharmaceutical compounds from a library of known drugs and biologically active chemical compounds, using information about known drugs from HumanPSD™ and predicting potential drugs using PASS program.

Method for analysis of known pharmaceutical compounds

We selected compounds from HumanPSD™ database that have at least one target. Next, we sort compounds using "*Drug rank*" that is the sum of the following ranks:

1. ranking by "Target activity score" (*T-score*$_{PSD}$),
2. ranking by "Disease activity score" (*D-score*$_{PSD}$),
3. ranking by "Clinical validity score".

"Target activity score" (*T-score*$_{PSD}$) is calculated as follows:

$$T\text{-}score_{PSD} = -\frac{|T|}{|T| + w(|AT| - |T|))} \sum_{t \in T} log_{10}\left(\frac{rank(t)}{1 + maxRank(T)}\right),$$

where *T* is set of all targets related to the compound intersected with input list, |*T*| is number of elements in *T*, *AT* and |*AT*| are set set of all targets related to the compound and number of elements in it, *w* is weight multiplier, *rank(t)* is rank of given target, *maxRank(T)* equals *max(rank(t))* for all targets *t* in *T*.
We use following formula to calculate "Disease activity score" (*D-score*$_{PSD}$):

$$D\text{-}score_{PSD} = \begin{cases} \sum_{d \in D} \sum_{p \in P} phase(d,p) \\ 0, \ D = \varnothing \end{cases},$$

where *D* is the set of selected diseases, and if *D* is empty set, *D-score*$_{PSD}$*=0*. *P* is a set of all known phases for each disease, *phase(p,d)* equals to the phase number if there are known clinical trials for the selected disease on this phase and zero otherwise.

The clinical validity score reflects the number of the highest clinical trials phase (from 1 to 4) on which the drug was ever tested for any pathology.

Method for prediction of pharmaceutical compounds

In this study, the focus was put on compounds with high pharmacological efficiency and low toxicity. For this purpose, comprehensive library of chemical compounds and drugs was subjected to a SAR/QSAR analysis. This library contains 13040 compounds along with their pre-calculated potential pharmacological activities of those substances, their possible side and toxic effects, as well as the possible mechanisms of action. All biological activities are expressed as probability values for a substance to exert this activity (*Pa*).
We selected compounds that satisfied the following conditions:

1. Toxicity below a chosen toxicity threshold (defines as *Pa*, probability to be active as toxic substance).
2. For all predicted pharmacological effects that correspond to a set of user selected disease(s) *Pa* is greater than a chosen effect threshold.
3. There are at least 2 targets (corresponding to the predicted activity-mechanisms) with predicted *Pa* greater than a chosen target threshold.

The maximum *Pa* value for all toxicities corresponding to the given compound is selected as the "Toxicity score". The maximum *Pa* value for all activities corresponding to the selected diseases for the given compound is used as the "Disease activity score". "Target activity score" (T-score) is calculated as follows:

$$T\text{-}score(s) = \frac{|T|}{|T| + w(|AT| - |T|))} \sum_{m \in M(s)} \left(pa(m) \sum_{g \in G(m)} IAP(g) optWeight(g) \right),$$

where *M(s)* is the set of activity-mechanisms for the given structure (which passed the chosen threshold for activity-mechanisms *Pa*); *G(m)* is the set of targets (converted to genes) that corresponds to the given activity-mechanism (m) for the given compound; *pa(m)* is the probability to be active of the activity-mechanism (m), *IAP(g)* is the invariant accuracy of prediction for gene from *G(m)*; *optWeight(g)* is the additional weight multiplier for gene. *T* is set of all targets related to the compound intersected with input list, |*T*| is number of elements in *T*, *AT* and |*AT*| are set set of all targets related to the compound and number of elements in it, *w* is weight multiplier.
"Druggability score" (D-score) is calculated as follows:

$$D\text{-}score(g) = IAP(g) \sum_{s \in S(g)} \sum_{m \in M(s,g)} pa(m),$$

where *S(g)* is the set of structures for which target list contains given target, *M(s,g)* is the set of activity-mechanisms (for the given structure) that corresponds to the given gene, *pa(m)* is the probability to be active of the activity-mechanism (m), *IAP(g)* is the invariant accuracy of prediction for the given gene.

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Supplementary material

1. Supplementary table 1 - Up-regulated genes
2. Supplementary table 2 - Down-regulated genes
3. Supplementary table 3 - Detailed report. Composite modules and master regulators (up-regulated genes in noRA_Dox vs. noRA_noDox).
4. Supplementary table 4 - Detailed report. Composite modules and master regulators (down-regulated genes in noRA_Dox vs. noRA_noDox).
5. Supplementary table 5 - Detailed report. Pharmaceutical compounds and drug targets.

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Decisions regarding care and treatment of patients should be fully made by attending doctors. The predicted chemical compounds listed in the report are given only for doctor's consideration and they cannot be treated as prescribed medication. It is the physician's responsibility to independently decide whether any, none or all of the predicted compounds can be used solely or in combination for patient treatment purposes, taking into account all applicable information regarding FDA prescribing recommendations for any therapeutic and the patient's condition, including, but not limited to, the patient's and family's medical history, physical examinations, information from various diagnostic tests, and patient preferences in accordance with the current standard of care. Whether or not a particular patient will benefit from a selected therapy is based on many factors and can vary significantly.

The compounds predicted to be active against the identified drug targets in the report are not guaranteed to be active against any particular patient's condition. GeneXplain GmbH does not give any assurances or guarantees regarding the treatment information and conclusions given in the report. There is no guarantee that any third party will provide a

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The analysis report contains information about chemical drug compounds, clinical trials and disease biomarkers retrieved from the HumanPSD™ database of gene-disease assignments maintained and exclusively distributed worldwide by geneXplain GmbH. The information contained in this database is collected from scientific literature and public clinical trials resources. It is updated to the best of geneXplain's knowledge however we do not guarantee completeness and reliability of this information leaving the final checkup and consideration of the predicted therapies to the medical doctor.

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