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ResearchArticle   
Anunsupervisedcomputationalpipelineidentifiespotentialrepurposable drugstotreatHuntington’sdiseaseandmultiplesclerosis   
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| article | info | abstract |
| *Keywords:*  Computationaldrugdiscovery Drugrepurposing  Networkpharmacology  Huntington’sdisease  Multiplesclerosis | | Drugrepurposingconsistsinidentifyingadditionalusesforknowndrugsand,sincethesenewfindingsarebuilt onpreviousknowledge,itreducesboththelengthandthecostsofthedrugdevelopment.Inthiswork,we assembledanautomatedcomputationalpipelinefordrugrepurposing,integratingalsoanetwork-basedanalysis forscreeningthepossibledrugcombinations.Theselectionofdrugsreliesbothontheirproximitytothedisease ontheprotein-proteininteractomeandontheirinfluenceontheexpressionofdisease-relatedgenes.Combined therapiesarethenprioritizedonthebasisofthedrugs’separationonthehumaninteractomeandtheknown drug-druginteractions.Weeventuallycollectedanumberofmolecules,andtheirplausiblecombinations,that couldbeproposedforthetreatmentofHuntington’sdiseaseandmultiplesclerosis.Finally,thispipelinecould potentiallyprovidenewsuggestionsalsoforothercomplexdisorders. |

**Introduction**

Discoveringanewdrugandbringingittothemarketisaprocess bothmoneyandtimeconsuming.Instead,relyingonestablisheddrugs, computationaldrugrepositioningoffersavaluablealternativeapproach forprovidingpromisingtreatmentsfordisorderswithoutacure[1,2]. Inrecentyears,aplethoraofcomputationalapproachestodrugrepur-posinghavebeenproposedandappliedtoawidevarietyoftherapeu-ticareas[3].Mostofsuchapproachesrelyeitheronmachinelearn-ingoronthetraditionalmethodsofcomputationaldrugdesign,even thoughsomeconceptuallyinnovativeideashavebroughttothelight thepossibilityoftakingnewpathstowardsthepredictionofpotentially repurposabledrugs.Oneofsuchideasisbasedonasystemviewand takesthehumanprotein-proteininteractomeasareferencenetworkto quantifytherelatednessbetweendrugsanddiseasesbycalculatingthe distancebetweendrugtargetsanddisease-associatedproteins.Thisdis-tancehasbeenproposedasasuitablemetricstomeasurethe"proximity" betweendrugsanddiseases[4].Recently,leveragingontheconceptof drug-diseaseproximity[5],noveldrugindicationsforthetreatmentof cardiovasculardiseases[5,6],cancers[7],COVID-19[8],Alzheimer’s disease[9]havebeenproposed,demonstratinghowanetwork-based approachcouldsuccessfullyassisttheselectionofdrugstoberepur-posed.

Inthiswork,weassembledanautomatedcomputationalpipelineby integratingarecentlydevelopedschemetoscreenrepurposabledrugs thatcombinesanetwork-basedtechniquewithananalysisofbiologi-

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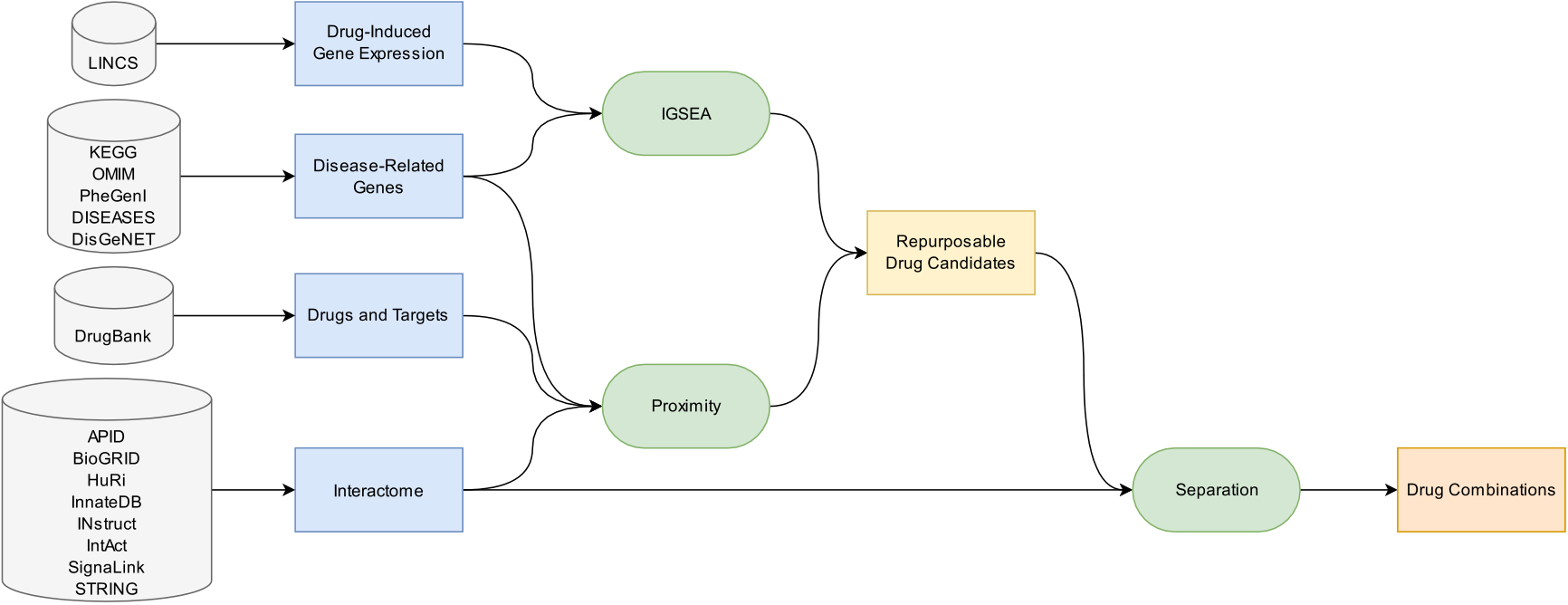
*E-mailaddress:*[maurizio.recanatini@unibo.it](mailto:maurizio.recanatini@unibo.it)(M.Recanatini).

calandexperimentaldata[10,11],withastrategyforfilteringallthe possibledrugcombinations[6].Initially,theprocedureestimatesthe proximitybetweenthedisease-relatedproteinsandthedrugtargetson theprotein-proteininteractome,performingafirstselectionofcandi-dates.Then,onlythosedrugsthatsignificantlyinfluencetheexpression ofdisease-relatedgenesareconsideredplausibleforrepurposing.Fi-nally,evaluatingtheseparationofthesedrugs’targetsonthehuman interactomeandtakingintoconsiderationtheknowndrug-druginter-actions,combinedtherapiesareprioritized.Theworkflowofthepro-cedureisschematicallyillustratedinFig.1.Theentireprocessisau-tomatedinordertoreducehumanintervention,thusacceleratingthe wholeprocedureandlimitingexecutionerrors.

WeappliedthispipelinetoHuntington’sdisease(HD)andmultiple sclerosis(MS)because,despitethefactthattheyarebothneurologi-caldisorders,theirdifferentnaturecouldrepresentachallengeforour strategy,andtheoutcomescouldgiveusinsightsintoitsmethodolog-icalstrengthsandlimitations.HD,isreportedasatypicalmonogenic disease,eventhoughmanyothergenesareknowntoinfluenceitspro-gression[12],whileforMSasinglegeneticcausehasnotbeenfound yet,probablybecausemanyfactorsplayanimportantroleintheetiol-ogy.Indeed,MSfitswellthedefinitionofcomplexdiseasetobeconsid-eredintheframeworkofnetworkmedicine.Ontheotherhand,HDwas includedinourstudyinordertotestthecapabilitiesoftheproposed methodinacasewheredifferentclinicalphenotypesmightberelated toadiseasemoduleeventuallyinfluencedbygeneticmodifiersleading todifferentpathophysiologicalstates[12–14].

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[2667-3185/© 2022TheAuthors.PublishedbyElsevi](http://creativecommons.org/licenses/by-nc-nd/4.0/)erB.V.ThisisanopenaccessarticleundertheCCBY-NC-NDlicense (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

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**Fig.1.**PipelineFlowchart.Theflowchartshowsthesourcesandthestepsoftheautomatedproceduretoscreenrepurposabledrugcandidatesandprioritizetheir

combinations.

HDisthemostcommonmonogenicneurologicaldisorder.Theon-setistypicallyintheearlystageofadultlife,anditischaracterized bymotordysfunction,cognitiveimpairment,andneuropsychiatricfea-tures[12,15].TheautosomaldominantmutationthatcausesHDislo-catedinthe*HTT*gene,anditconsistsinacytosine-adenosine-guanine trinucleotiderepetition(CAG,encodingglutamine)leadingtoanover-expansionofthepolyglutamine(polyQ)tailinthehuntingtinprotein. Themutatedproteintendstoaggregateandaccumulate,forminginclu-sionbodiesthathavedeleteriousconsequencesfortheneuralcell.Both theinclusionbodiesandthelengthoftheCAGexpansionareproven toplayanimportantroleinthedevelopmentofthedisease.Theclear-anceofthefirstonesslowstheHDprogression,whilethelongerthe CAGexpansion,theearlierthediseasemaymanifest[15].Theremain-inguncertaintyonthecourseofHDcanbeascribedtoothergenetic differencesinthegenomeofthepatients[12,14].

MSisboththemostfrequentnon-traumaticdisablingdiseasein youngadults[16]andthecommonestdemyelinatingdisease[17].The etiologyandthemechanismcausingitsworseningprogressionarestill unclear,neverthelessithasbeenproventhatacomplexinterplayofge-neticandenvironmentalfactorsisimportant[18,19].Themainknown riskfactorsaresmoking,childhoodobesity,infectionwiththeEpstein-Barrvirus,andlowvitaminDlevels[19].MSisgenerallyviewedasa two-phasesautoimmunedisease,inwhichinitiallyfocalinflammatory processescausearelapsing-remittingformofthedisease,andsubse-quentlydemyelinatingplaques(lesionsresultedbythepreviousimmune response)andoligodendrocytedamageleadtoneurodegenerationand non-relapsingprogressivecourse[17,19].MSiscommonlycharacter-izedbyprogressivespasticparaparesis,cognitiveimpairment,andsen-soryandcerebellardysfunctions[19].

BothHDandMSarestilllackingresolutivetreatments[20,21], whosedevelopmentneedsadeeperknowledgeoftheunderlyingmech-anisms[22].Tothisaim,network-basedmodels,astheonesweutilized inthisstudy,couldbeadequatetheoreticaltoolsforinvestigatingsuch multifactorialdisorders.Theywouldallowustotakeintoaccountthe latentcomplexstructureofthesediseaseswithoutlosingacomprehen-siveview[23].Throughthemethodologypresentedhere,wewereable tocollectanumberofapproveddrugsandtheirplausiblecombinations thatcouldbeproposedforthetreatmentofHDandMS.

**Materialsandmethods**

Theworkflowofthisstudycanbeoutlinedinthefollowingsteps (Fig.1):(1)collectionofdisease-relatedgenes;(2)genesetsvalidation throughenrichmentanalysis;(3)collectionofdrugs,targets,protein interactiondata,andconstructionofprotein-proteininteractome;(4)

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*Drugsandtargetscollection,geneexpressionprofilesretrievaland protein-proteininteractomeconstruction*

DrugsinformationwascollectedfromDrugBank[33](version 5.1.9).Onlythosemoleculeshavingatleastonehumanproteinastarget areconsidered,obtaining5798drugsand2755correspondingtargets. Drug-inducedgeneexpressionprofileswereretrievedfromtheLi-braryofIntegratedNetwork-basedCellularSignatures[34](LINCS,pro-files“GSE70138” and“GS[E92742”),downloadedfromGen](http://www.ncbi.nlm.nih.gov/geo)eExpres-sionOmnibus[35](GEO,<http://www.ncbi.nlm.nih.gov/geo>).Dueto thefactthatweareinspec[tingneurologicaldisorders,thoses](http://www.ncbi.nlm.nih.gov/geo)ignatures testedonneuralcelllines(NEU,NPC,SHSY5Y)wereexaminedforboth diseases.Additionally,inordertoconsiderdisease-specificfeatures,also muscularcelllines(SKB,SKL)wereincludedforHD,andhaematopoi-eticandlymphoidtissuecelllines(L60,JURKAT,NOMO1,PL21,SKM1, THP1,U937,WSUDLCL2)forMS.Furthermore,toguaranteemaximum reliabilityoftheresults,onlythedataabouttheBestInferredGenes (BING)ineverydataset(drugsignature)intheseprofileswaskept.The BINGsubsetincludes978landmarkgenesand9196inferredgenes, whichareidentifiedamongthe12328genesintheL1000assayby Subramanianetal.[36]evaluatingthemostreliableinferencepredic-tions.

Extensiveinteractionsamongproteinsareakeyfactorinaccom-plishingmanybiologicalprocessesandfunctions.Forthisreason, weoptedforanetwork-basedapproachtoevaluatethecorrelation betweendrugsanddiseasesordrugsandotherdrugs.Webuiltahuman protein-proteininteraction(PPI)networkcombiningdatafromeight publiclyavailable[resources:AgileProteinInteractomesDataServe](http://cicblade.dep.usal.es:8080/APID/init.action)r [37] (APID,   
 <http://cicblade.dep.usal.es:8080/APID/init.action>), Biologica[lGeneralRepository](https://thebiogrid.org/)[forInteractionDatasets[38](B](http://cicblade.dep.usal.es:8080/APID/init.action)i-oGRID,<https://thebiogrid.org/>[),TheHumanRefere](http://www.interactome-atlas.org/)nceInterac-[tome[3](http://instruct.yulab.org/)[9](HuRI,http://ww](https://thebiogrid.org/)[w.interactome-atlas.org/),InnateDB [40](https://www.innatedb.com/),INstruct[41](http://instruct. yulab.org/),IntAct[42](https://www.ebi.ac.uk/intact/home),Sig-naLink[43](http://signalink.org/),andSearchToolfortheRetrieval](http://instruct.yulab.org/) ofInteracting[Genes/Proteins[44](http://signalink.org/)](STRING,<https://string-db.org/>). SupplementaryTable1givesadditionalinfo[abouttheinteraction](https://string-db.org/)s reportedinthedatabasesandtheappliedfilters.

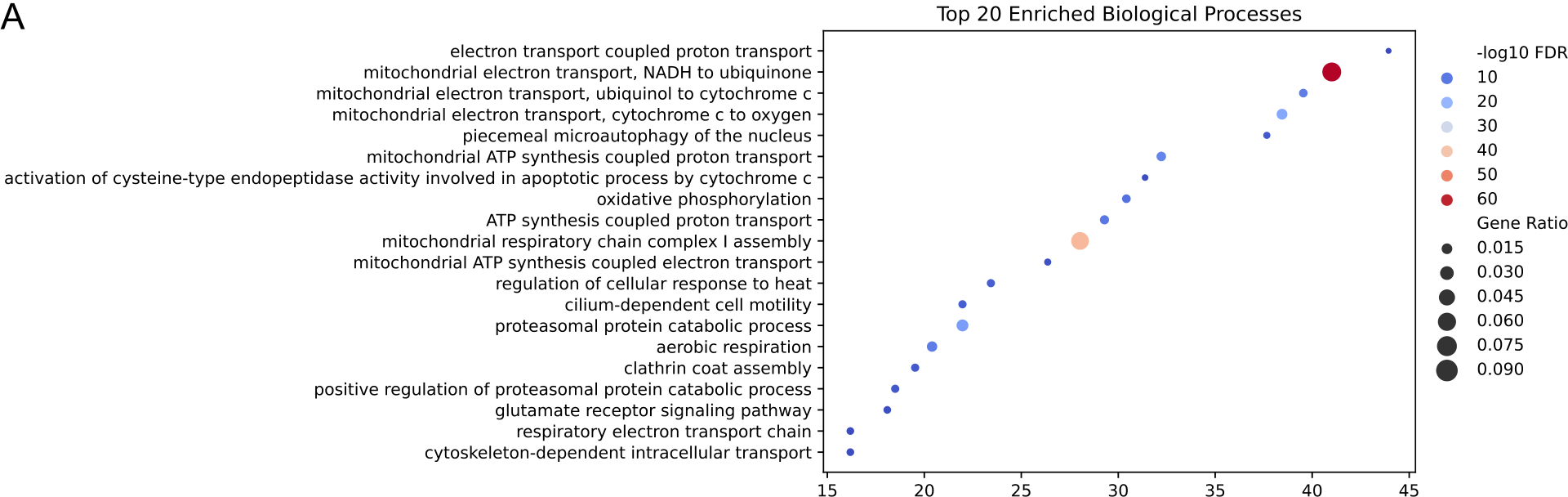
Theretrievedinteractionswerethencombined,obtaininganet-work(availableintheSupplementaryInformation)consistingof20445 nodes(genes/proteins)and1125173edges(interactions).Consistency isgrantedbythefactthatalllistedproteinsaremappedtoofficial genesymbolstakingadvantageoftheNCBIdatabase.Sincetheprotein-proteininteractomeisthesupportingpillarofthewholeprocedure,we assesseditsvaliditycomparingtheresultsoftheentireanalysesbased ontwootherinteractomes.Thefirstrerunwascarriedoutonthewidely recognizedinteractomefromChengetal.[5](16677uniqueproteins and243603experimentallyconfirmedprotein–proteininteractions). Thesecondonewasperformedonadrasticallyrestrictedversionofour owninteractome(16954proteinsand246080interactions),inwhich onlyinteractionsfromlowthroughputstudies(listinglessthan20in-teractions)wereincluded.

*Networkproximity*

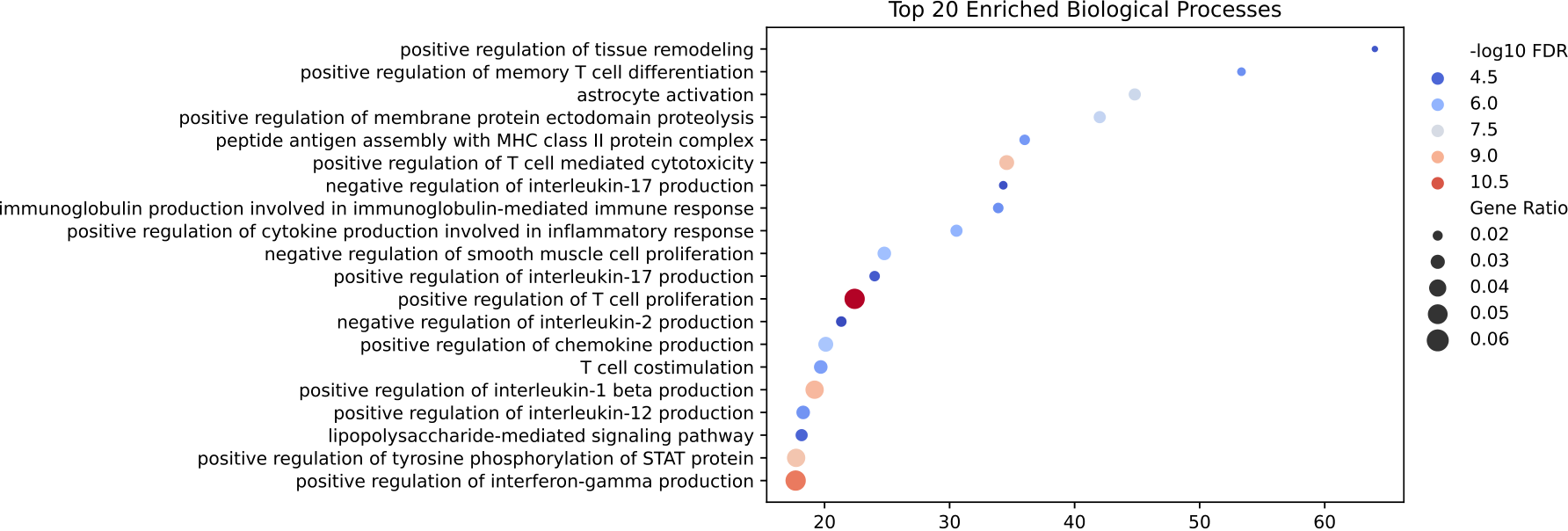
Proteinsrelatedtoaspecificdiseaseareunlikelytobescattered throughouttheinteractome,rather,theytendtogrouptogetherforming theso-calleddiseasemodule[45].Therelationshipbetweenadrugand adiseasecouldbeestimatedbymeansofanunsupervisedandunbiased network-basedapproach[4],whichquantifiestheinterplayofdrugtar-getsanddisease-relatedgenesmeasuringanetworkproximity.Herewe usedarecentlymodifiedversionofsuchmethod[10]thatincludesa term(*𝑤*)fortakingintoaccountthedegreeofthedrugtargetsdirectly intothedistancecalculation.Given*𝐺*,thesetofdisease-relatedgenes; *𝑇*,thesetofdrugtargets;and*𝑑*(*𝑔,𝑡*),theshortestpathlengthbetween nodes*𝑔* (*𝑔*∈ *𝐺*)and*𝑡*(*𝑡*∈ *𝑇*)inthehumanprotein-proteininteractome;

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**Fig.2.**EnrichedBiologicalProcesses.Thebubbleplotsdisplaythetop20mostenrichedGeneOntologytermsrelativetobiologicalprocessesforHuntington’sdisease (A)andmultiplesclerosis(B).Onthehorizontalaxis,thefoldenrichmentisshown.Thecolorencodesthenegativeofthefalsediscoveryratelogarithm,andthe sizerepresentsthegeneratio(computedastheratioofthepercentageofgenesinthestudysetrelatedtoaspecificterm,dividedbythecorrespondingpercentage inthebackground,i.e.,theentirehumanproteome).

**Results**

*Computationalframework*

Inthisstudy,weautomatedthepipelineshowninFig.1forscreening repurposabledrugcandidatesandprioritizingtheircombinations.Inor-dertorun,thescriptonlyrequiresthediseasename,thedisease-related genes,andthecelllinesofinterestasinputs.Thisprocedureconsists of:collecting,cleaningandorganizingthesourcedata(disease-related genes,drugs,targets,proteininteractions,drug-inducedgeneexpres-sionsignatures);identifyingrepurposabledrugcandidatesevaluating boththeirproximitytothediseaseandtheireffectontheexpressionof thedisease-relatedgenes;screeningthepossibledrugcombinationson thebasisoftheirrelativeexposureandknowninteractions.Theoutput oftheroutineisacollectionoftables(tab-separatedvaluesfiles)and plots,recordingbothintermediateandfinalresults.

Comparedtopreviousrelatedworks[10,11],suchasystematicstrat-egyshouldbemoreefficientandhaveanimprovedreproducibility thankstotheorganizationandstandardizationofboththeoverallstudy andresults.Additionally,ittakesastepforwardsinceitevaluatesalso possiblecombinedtherapies.

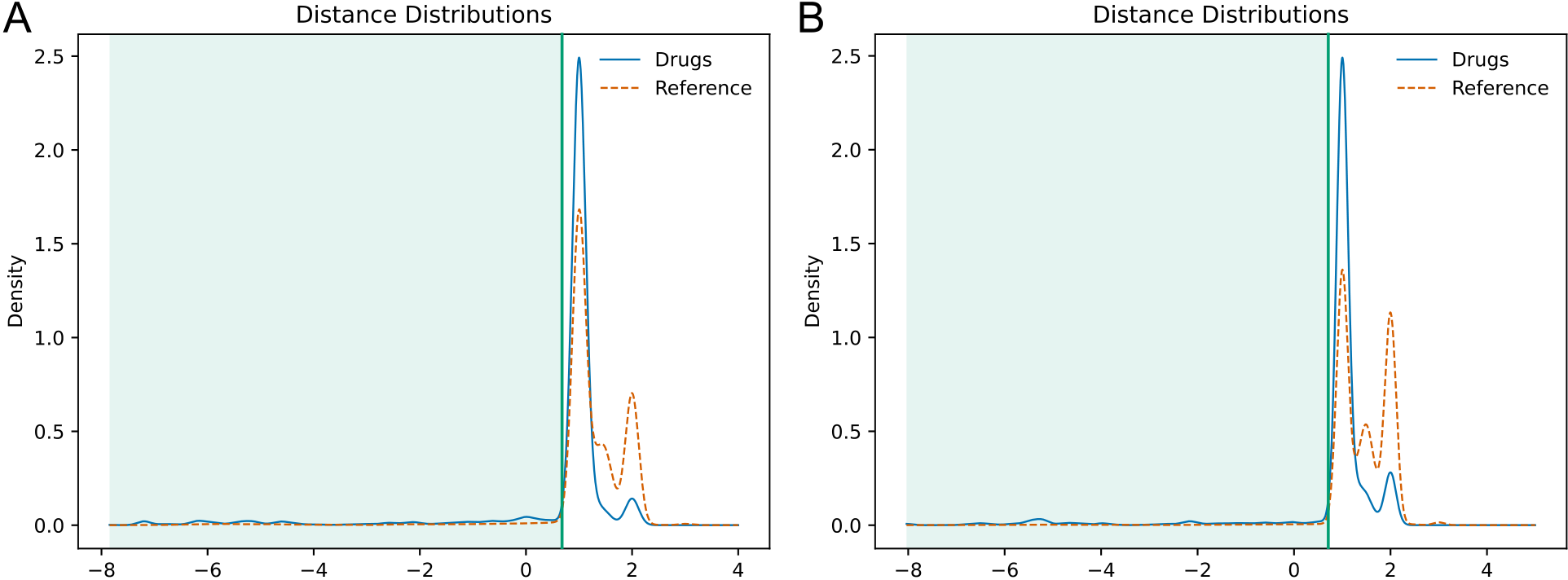
Thesinglestepsandtheoutcomesoftheapplicationoftheframe-worktoHDandMSarepresentedanddiscussedinthefollow-ing.

*Disease-relatedgenescollectionandvalidation*

Wegatheredthedisease-relatedgenesasdescribedintheMethods section:thisresultedin451and217genesassociatedtoHDandMS,

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**Fig.3.**DistanceDistributions.Thedistributionofthedistancebetweendrugtargetsanddisease-relatedproteins(solidblueline)comparedtothatofareference

collection(dashedorangeline),forHuntington’sdisease(A)andmultiplesclerosis(B).Ontheverticalaxis,thekerneldensityestimationofthedistributionisshown.

Theplotisdividedintotwopartsbythechosendistancethreshold(greenline,seeMethods).

*Repurposabledrugsselection*

Thenetwork-basedproximityanalysis,leveragingonthepotential ofasystemview,couldsuggestvaluabledrugsabletointerferewith thediseasemoleculardeterminantsinanon-trivialway(i.e.,notonly directlytargetingdisease-relatedgenes).Theideabehindthismethod isthatdrugsproximaltothediseasemoduleshouldbemoreeffec-tivethandistantones,asshownbyGuneyetal.inanextensiveanal-ysisthatconsideredknowndiseasesanddisease-associatedgenes,as wellasdrugsandtheirtargets[4].FollowingPeng’sprotocol[10], theprocedurecomparesthedistributionofthedistancesbetweendrug targetsanddisease-relatedproteinstothatofareferencecollection (seeMethodsandFig.3).Forbothdiseases,itwaspossibletoiden-tifyadistancevaluebelowwhichthetwodensitycurves(drugsand reference)dropdramatically.Inparticular,thereferencedensityas-sumesnegligiblevaluesfordistancesbelowthispoint(Fig.3,green partoftheplot).Weelectedsuchdistancevalue(Fig.3,vertical greenline)asthethresholdtodiscriminatedrugsassociatedtothedis-eases.Thesedistancesare0.68and0.71(correspondingtoproximity:−0.53and–0.98)forHDandMS,respectively.Fromthisanalysis,685 (11.8%)outofthe5798drugscollectedfromDrugBankwereconsid-eredsignificantlyproximalmedicamentsforHD,and475(8.2%)for MS.

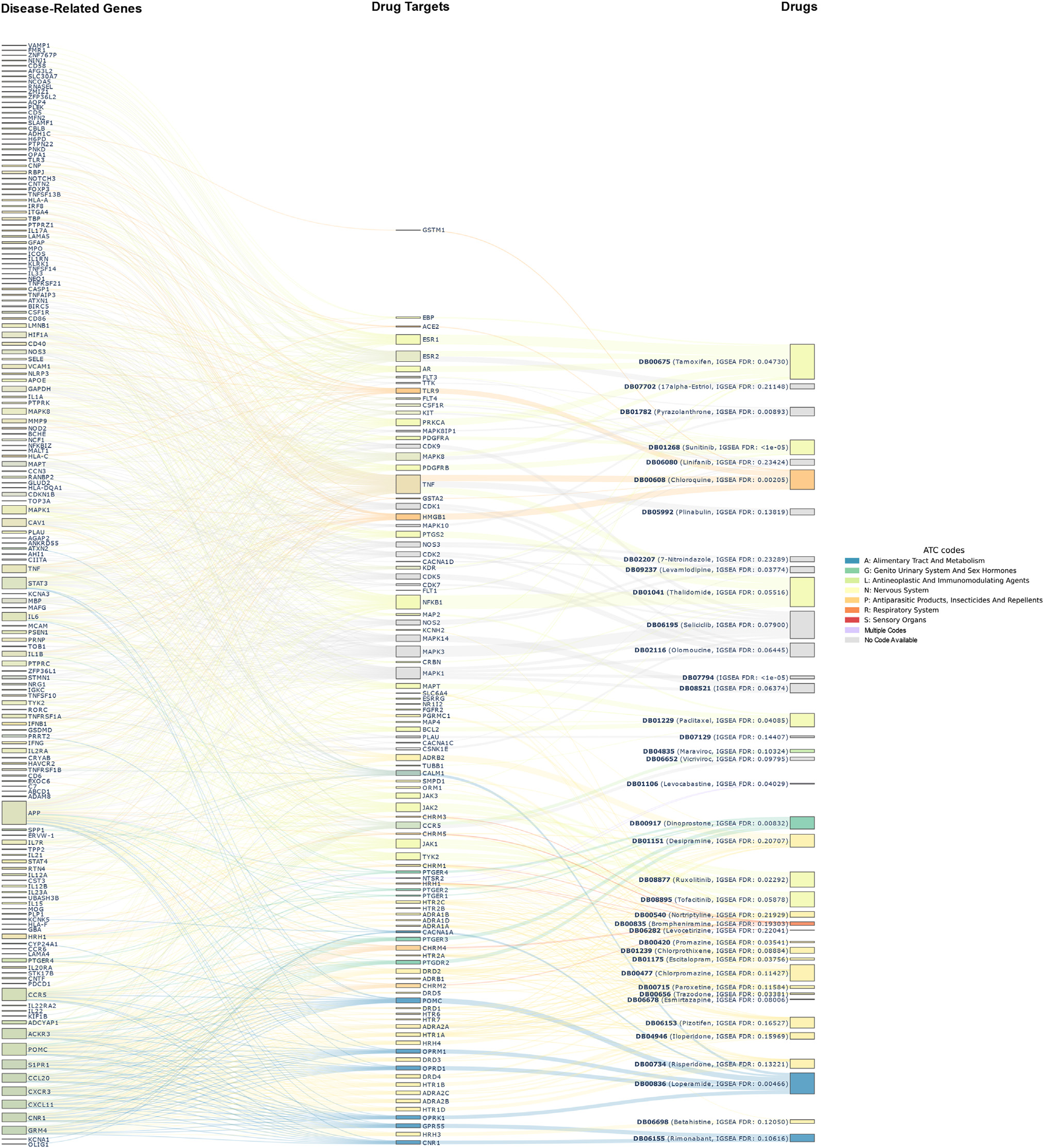
Inordertoevaluatetheimpactofadrugonthedisease,weexamined theeffectofitsadministrationontheexpressionofthedisease-related genesinrelevantcelllines(seeMethods).Wepursuedthisobjective byperforminganInvertedGeneSetEnrichmentAnalysis(IGSEA)on 896drugs,observedin6212LINCSexpressiondatasetsforHDand960 drugsin5579datasetsforMS.Thisanalysisresultedin843and600 significantlyenricheddrugs,forHDandMSrespectively.

Thedrugsthatwerebothsignificantlyenrichedandproximalto thediseaseweredeemedtoberepurposabledrugcandidates:138for HDand38forMS(SupplementaryTables2,3).Theinteractionsbe-tweentheMS-related-genes,thedrugtargets,andtherepurposabledrug candidatesarevisualizedinFig.4(andSupplementaryFigure4for HD),showinghowdrugscanberelatedtothediseasethroughtheir targets.

Unfortunately,onlyasmallportionoftheproximaldrugshasdata intheLINCSdatabase(21.9%forHDand13.7%forMS).Eventhough theIGSEAanalysisincreasesthereliabilityoftheresults,itdramatically reducesthenumberofmoleculesthatcanbeinvestigatedandpossibly proposed.Thishastobetakenintoaccountwhenevaluatingtheout-comesofthestudy.

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**Fig.4.**MultipleSclerosisGene-Target-DrugNetwork.TheSankeydiagramillustratestheinterconnectionsbetweendisease-relatedgenes,drugtargets,anddrugs.

Eachdrug(rightcolumn)isconnectedtoitsreportedtargets(middlecolumn),which,inturn,areproximalonthehumaninteractometosomeofthedisease-associated

proteins(leftcolumn).DrugsarecoloredbytherespectiveATCcode,andtheFDRoftheIGSEAanalysis(seeMethods)isreportedinthelabel.

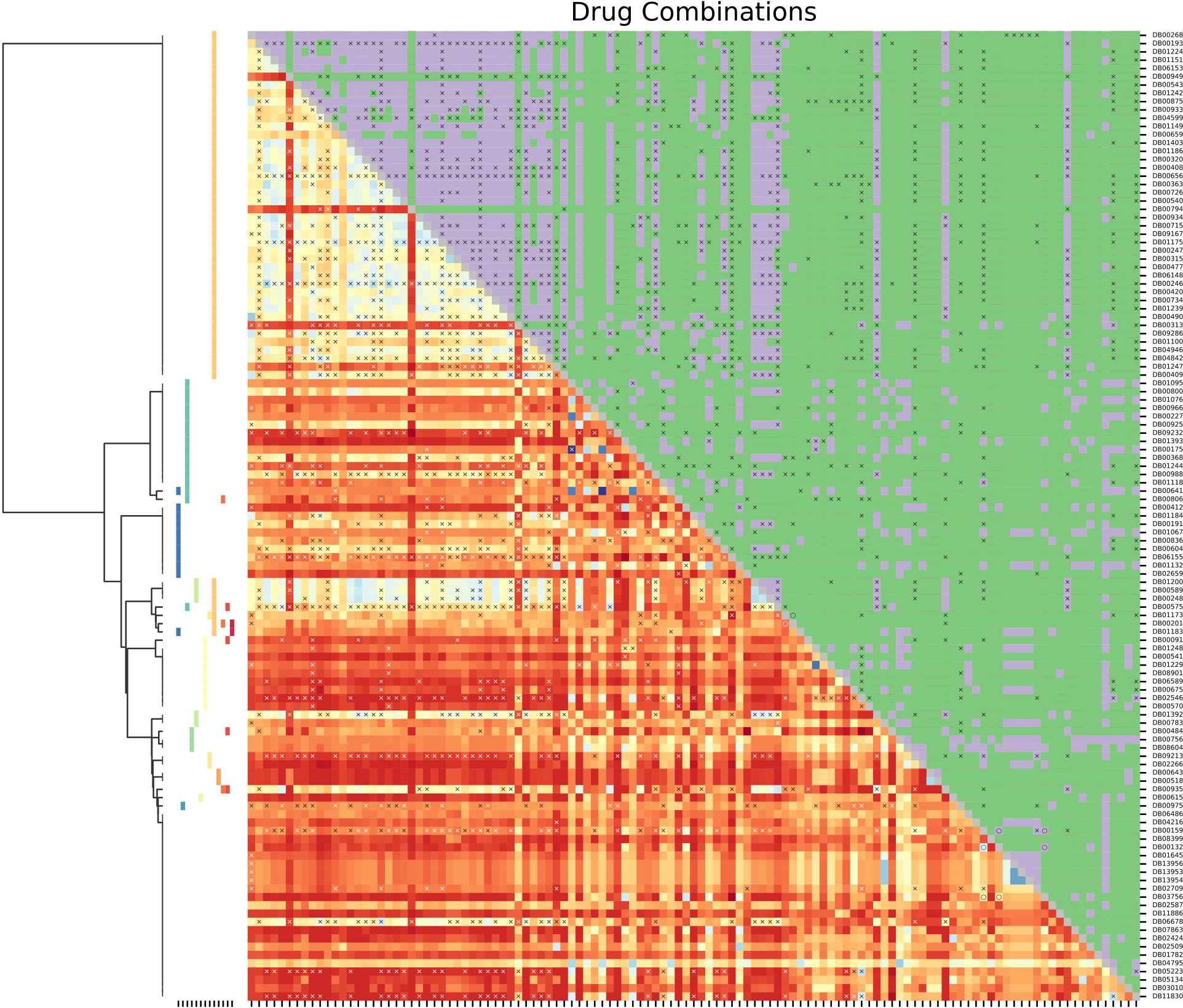
creasedperformance,whichisperfectlyinlinewiththeBigDataper-spective[47].

*Repurposabledrugs*

AmongthedrugsselectedtotackleHD(138),several(17)havebeen clinicallytestedandsuggested,manyshowstrongevidencefrominvivo tests(35)orpromisingresultsfrominvitroassays(9).Allthereferences arereportedintheSupplementaryTable2,themostnoticeableexam-

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**Fig.5.**Huntington’sDiseaseDrugCombinations.Theannotatedheatmapprovidesinfoaboutpossiblecombinationsoftheselecteddrugs.Acombinationismarked with× ifaninteractionisreportedinDrugBank,andwith○ ifitispresentinanapprovedformulation.Thelower-leftpartoftheheatmapshowstheseparation oftheinspecteddrugs,colorcodedfromblue(noseparation)tored(stronglyseparated).Theupper-rightportion,instead,displaysthekindofexposure:violetif overlappingandgreenifcomplementary.Attheleftmostpart,theATCcodesofthedrugsarereportedalongwithadendrogramoftheirhierarchicalclustering.

hasbeneficialeffectsinthetreatmentofpsychiatricmanifestationsand stabilizationofmotorsymptomsinpatientswithHD[20].

InspectingthedrugsscreenedforMS(38),weobtainedacomparable outcome:7ofthemareclinicallystudiedand9experimentedonanimal models.AlltheevidenceislistedintheSupplementaryTable3.Most

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ExaminingtheAnatomicalTherapeuticChemical(ATC)codesofthe repurposabledrugs,thefirstthingtonoticeisthepredictablepreva-lenceofdrugsassociatedtotheATCcodeN(NervousSystem)forboth diseases.Apartfromthis,themostcommoncodesforHDrepurposable drugsareC(Cardiovascular)andL(AntineoplasticandImmunomodu-latingAgents).Thefirstgroupismainlyrepresentedbystatins,usedto copewiththecholesterolimpairmenttypicalofHDpatients[52].The immunomodulatingagentsareprincipallyimmunosuppressantsandhi-stonedeacetylasesinhibitors,thelastonesaimedatrecoveringfromthe histonehypoacetylationcommoninneurologicaldisorders[53]. ForMS,instead,thesecondmostfrequentcodeisL(Antineoplastic andImmunomodulatingAgents).Somerelevantexamplesareruxoli-tinib,paclitaxel,tamoxifen,andthalidomide,whicharecapableofat-tenuatingexperimentalautoimmuneencephalomyelitisandofinducing remyelination[54–58].

*Drugcombinations*

Observingtheobtainedresults(depictedasannotatedheatmapsin Fig.5forHDandSupplementaryFigure5forMS)itisinterestingto

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highlightthatdrugsthatdonothaveATCcodesassociatedtothemare alsothosewithfew(ornothingatall)reportedinteractions.Thissug-geststhattheyarenotsufficientlycharacterizedandadditionalstudies onthemareneededbeforefurtherconsideration.

Thecollectedplausiblecombinationsarenumerous,buttheassoci-ationoforphenadrine(DB01173)andcaffeine(DB00201)forHDde-servestobehighlighted.Thesemoleculesarepresentalongwithacetyl-salicylicacid(ASA)inanFDAapprovedformulationformuscularpain relief.Thismedicationisnoteworthyformanyreasons.Firstofall, painisaknownissueinHDandcouldbeanimportantnon-motor symptom[59,60]thus,itstreatmentshouldnotbeneglected.Further-more,orphenadrineshowedtobeeffectiveinpreventingneurotoxicity inratswithachemically-inducedconditionthatmimicsthehistologi-calandneurochemicalfeaturesofHD[61].Additionally,lowdosages ofcaffeineshowedtobebeneficialinHDanimalmodels[62].Finally, ASAwasincludedintheformulationforrelievingpainanddecreasing swelling.EventhoughASAwasproximaltoHD,itwasnotincludedin ourresultsbecauseitsdatawasnotavailableinLINCSfortheinvesti-gatedcelllines.However,itisactuallyprofitableforthepresentaim, sinceitshowedtopreventproteinaggregationinseveralneurodegen-

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|  | **Fig.6.**EscitalopramandTofacitinibComple-mentaryExposure.Thenetworkdisplaysthe proteinsassociatedtoMS(circles)andhigh-lightsthosetargetedbyescitalopramandtofac-itinib(darkgreenanddarkblue,respectively). TargetsthatarenotrelatedtoMSareindicated astriangles.Inordertobetterillustratethein-fluenceofthesetwomoleculesgivenbythe tightinterconnectionoftheproteome,thefirst neighborsofthedrugtargetsaredepictedin alightercolor(lightgreenforneighborsoftar-getsofescitalopram,andlightbluetofacitinib’s ones). |

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erativediseases[63].Furtherassessmentsareneeded,butthiscouldbe aninterestingpointwheretostart.

AreasonablehypothesisfortreatingMSmightbeanassociation oftwodrugssufficientlyseparatedfromeachotherasescitalopram (DB01175)andtofacitinib(DB08895)orruxolitinib(DB08877),capa-bleofaffectingcomplementarypartsofthediseasemodule.Fig.6shows thenetworkoftheinteractionsamongproteinsassociatedtoMS(allcir-cles)andhighlightsthosetargetedbyescitalopramandtofacitinib(dark greenanddarkblue,respectively).Amongthesetargets,twoofthem, namelyHRH1forescitalopram(darkgreencircle)andTYK2fortofac-itinib(darkbluecircle),belongtotheMSdiseasemodule,whilethose thatarenotdirectlyrelatedtoMSaredepictedastriangles(maintain-ingthesamecolorcoding).Inordertobetterillustratetheinfluenceon thediseasemoduleofthetwodrugsintermsofprotein-proteininter-actions,thefirstneighborsofthedrugtargetsarecoloredlighter(light greenforneighborsoftargetsofescitalopram,andlightbluefortofaci-tinib’sones).Itcanbeseenthatoverallthetargetsofbothescitalopram andtofacitinibortheirfirstneighborscaninfluenceareasonablepartof thediseasemodulewithoutredundantlyinterferingwiththesameMS proteins.Infact,ouranalysisshowedthatthesedrugsareproximalto MSandsignificantlyinfluenceproteinsassociatedtothisdisease.Addi-tionally,nointeractionsbetweenthemhavebeenreportedinDrugBank. Moreover,wefoundexperimentalevidencesupportingthisinference. Escitalopramisaselectiveserotoninre-uptakeinhibitor(ATCcode:N, Nervous)thatinhumansprovedtopreventstress-relatedrelapses[51]. Tofacitinibandruxolitinibshowedpromisingeffectsinanimalmodels: thefirstoneenhancingremyelinationandimprovingmyelinintegrity [64],andthesecondoneamelioratingtheseverityofthedisease[54]. Furthermore,theyareJanuskinase(JAK)inhibitors(ATCcode:L,Anti-neoplasticandImmunomodulatingAgents)andtheJAK/STATpathway isaberrantlyactivatedinMS[21,65].

Intheotherdrugcombinations,whicharesufficientlyseparated(see Methods,greenontheheatmaps)andforwhichnoadverseinteractions arereported(notannotatedwithan× intheheatmaps),valuableclues forpolypharmacologicalinterventionscouldbefound.Aworkinghy-pothesismightbetochoosetwodrugstacklingdifferentaspectsofa disease,forinstancefeaturingdistinctATCcodes.

**Limitations**

Despiteourbestefforts,thisstudyisnotexemptfromsomeshort-comingsthatarecommonindataanalysis,andregardmainlythedata availabilityandquality.Thiscouldhaveledustomisssomepromis-ingcompoundsand,atthesametime,itmaycompromisesomeofthe analyses.

Acompletecharacterizationofallavailabledrugsandhumanpro-teinsissurelynotathand,andthishasrepercussionsonmanyaspects ofthestudy,like,e.g.,thehumanprotein-proteininteractomecon-struction,drugassociationtobiologicalprocesses,cellularcomponents, molecularfunctionsandphenotypes,anddruginducedgeneexpression profilesretrieval.Onlysometimes,thisissuecouldbepartiallymitigated byanextensiveintegrationofdatafromawidervarietyofdatabases. Noteworthy,puzzlingexamplescouldbethedrug-targetassociationand theavailabilityofexpressiondatainLINCS.Thenumberoftargetsas-sociatedtoaspecificdrugcouldconsiderablydependontheamount ofresearchcarriedoutonthatmedicineratherthanontheactualbi-ologicalinteractionsithas.Thisinfluencesthedrug-diseaseproximity evaluation.Additionally,asstatedabove,theLINCSdatabasedoesnot provideexpressionprofilesforallthedrugsselectedbynetworkprox-imity,limitingbyfarthechoicespacefordrugrepurposing.

Furthermore,iftheknowledgewehaveaboutdrugsisincomplete, theonewehaveontheircombinationisevensparser.This,obviously, affectsourabilitytoscreenandjudgeplausibleassociations.

Moreover,itcouldbearguedthat,eventhoughthedrug-disease proximityisevaluatedwitharigorousgeometricalapproach,thechoice

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