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IDENTIFICAÇÃO DE FATORES DE TRANSCRIÇÃO QUE AGEM COMO REGULADORES MESTRES DAS DOENÇAS DE PARKINSON E ALZHEIMER

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Co-orientador: Marco Antônio De Bastiani

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Índice

I. Resumo

As doenças de Alzheimer e de Parkinson são as enfermidades neurodegenerativas mais comuns atualmente. Em conjunto, essas duas patologias acometem mais de 45 milhões de pessoas no mundo inteiro e são responsáveis pela grande maioria dos casos de demência. Apesar do investimento em pesquisas na área, a etiologia e os mecanismos moleculares envolvidos na patogênese dessas doenças ainda não foram totalmente elucidados e não existem fármacos capazes de retardar ou impedir a morte neuronal característica dos indivíduos afetados. Mutações em genes específicos vêm sendo associadas a uma pequena parcela dos casos das doenças de Parkinson e Alzheimer. Porém, as vias em que estes genes mutados estão envolvidos e a interação com outros fatores que levam a processos patológicos ainda não são claras. Redes regulatórias específicas das doenças, inferidas a partir de algoritmos de engenharia reversa, podem melhorar a nossa habilidade de caracterizar perturbações sistemicamente. No presente trabalho, foram utilizados dados de microarranjo adquiridos da plataforma pública *Gene Expression Omnibus* (GEO) (GSE60862) para modelar redes de regulação transcricionais específicas para o hipocampo e a *substância nigra*, principais regiões danificadas nas doenças de Alzheimer e Parkinson. A seguir, estudos caso-controle (GSE5281 e GSE8397) foram usados para estabelecer assinaturas gênicas para as duas doenças neurodegenerativas de interesse e identificar fatores de transcrição (do inglês *transcriptionl factors* – TFs) cujos *regulons* encontram-se diferencialmente expressos em cada uma dessas situações patológicas, isto é, fatores de transcrição que são os reguladores mestres responsáveis pela modulação fenotípica que resulta na doença. Para tanto, foram realizadas duas análises, RMA (*Master Regulator Analysis*) e GSEA (*Gene Set Enrichment Analysis*), e apenas TFs identificados como Reguladores Mestres (*Masters Regulators* – MRs) por ambas foram considerados no estudo. Como resultado, identificamos 117 TFs importantes para a regulação da expressão gênica no hipocampo e 123 na *substância nigra*. Propusemos 17 MRs envolvidos com a doença de Alzheimer e 28 com

a doença de Parkinson, alguns dos quais já haviam sido descritos anteriormente em processos relacionados à neurodegeneração (como YY1, HMG20A, RREB-1 and SLC3A9). Esses resultados são de grande valor para a caracterização molecular das estruturas hipocampo e *substância nigra* e determinação das mudanças de padrão de expressão que ocorrem nesses tecidos quando afetados pelas doenças de Alzheimer e Parkinson. A análise e validação dessas assinaturas podem contribuir para elucidar o processo de neurodegeneração e fornecer dados para a busca de novos alvos terapêuticos.

II. Introdução

1. Doença de Alzheimer

1.1. Epidemiologia

A Doença de Alzheimer (DA) é a doença neurodegenerativa mais comum atualmente, responsável por cerca de 70% dos casos de demência [1]. Ela foi descrita pela primeira vez em 1906, pelo psiquiatra alemão Alois Alzheimer, que estudava o caso de uma mulher que apresentava uma forma "peculiar" de demência aos 51 anos. O pesquisador correlacionou danos cognitivos e mudanças comportamentais da paciente com achados histopatológicos após sua morte, descrevendo os principais componentes neuropatológicos da doença. A DA caracteriza-se pela morte gradual de neurônios em determinadas áreas do cérebro (principalmente camada piramidal do hipocampo, camada II do córtex entorrinal e certas áreas do neocortex temporal, parietal e frontal) levando inevitavelmente à morte dos pacientes em até 10 anos após seu diagnóstico. Danos na memória recente são, geralmente, os primeiros sintomas da DA. Eles podem ser acompanhados de outros déficits cognitivos, como perda da capacidade de resolver problemas e dificuldade atencional. À medida que a doença avança, prejuízos na fala, dificuldade visoespacial e mudanças na personalidade (falta de iniciativa, depressão, entre outros) frequentemente passam a ser manifestados pelos pacientes [1].

O principal fator de risco para a DA é a idade. Essa enfermidade acomete, em geral, pessoas maiores de 60 anos e sua prevalência aumenta exponencialmente com o passar do tempo. Após os 85 anos, a probabilidade de um indivíduo ser diagnosticado com a doença é maior que 33% [2]. O número alarmante de casos da doença tende a tornar-se ainda maior no futuro, uma vez que a expectativa de vida da população vem aumentando com o desenvolvimento da medicina. Assim, calcula-se que o número de indivíduos com a DA, em 2040, será três vezes maior que nos dias de hoje, chegando a 90 milhões [3].

Através de estudos epidemiológicos, muitos fatores de risco já foram descobertos para a DA, ainda que a forma exata como alguns deles agem continue desconhecida. Dentre estes fatores pode-se citar o sedentarismo, fumo, problemas cardiovasculares, diabetes tipo II e dislipidemias [4].

1.2. Características Neuropatológicas

Macroscopicamente, é comum a ocorrência de atrofia cortical, principalmente nos lobos temporais, fazendo com que os ventrículos laterais pareçam anormalmente dilatados [5]. Microscopicamente, as características principais são a presença de placas amilóides no meio extracelular e emaranhados neurofibrilares no interior dos neurônios. Morte neuronal, distrofia dos neuritos, perda sináptica, redução de certos neurotransmissores, danos oxidativos e inflamação (proliferação de astrócitos e microglia) também estão presentes [1]. As principais características neuropatológicas da doença estão ilustradas na **Figura 1**.

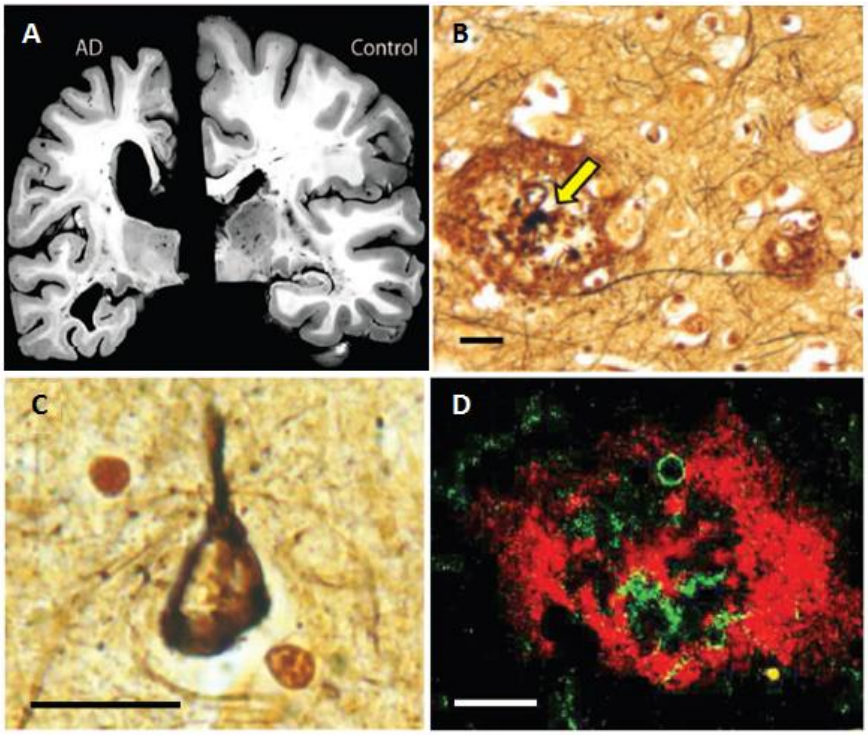

Figura 1: Características neuropatológicas da DA. (A) Comparação entre fatias de cérebros *post-mortem* de indivíduos com (à esquerda) e sem a doença (à direita), demonstrando severa atrofia. Cortes histológicos em que se pode identificar (B) placas amilóides e (C) emaranhados neurofibrilares corados com prata. Em (D), uma placa amilóide marcada com anticorpo anti-Aβ (em vermelho), mostrando

infiltrado de microglias, coradas em verde. (Adaptado de Holtzman et al., 2011 e O'Brien et al., 2011) [1, 6]

As placas amilóides são compostas majoritariamente pelo peptídeo β-amilóide (Aβ). Este é formado naturalmente no organismo e consiste em uma cadeia de 36 a 43 aminoácidos derivados da clivagem de uma proteína maior, chamada proteína precursora amilóide (APP) [3]. O processo de clivagem e formação do peptídeo está ilustrado na **Figura 2**. O Aβ é relativamente abundante no fluído cefalorraquidiano que banha o sistema nervoso central, sendo encontrado em concentrações de 10-20 ng/mL. O aumento dessa concentração, ou da concentração relativa de Aβ42 (principal fragmento presente nas placas amilóides) implica na formação de agregados e patogênese da Doença de Alzheimer [1].

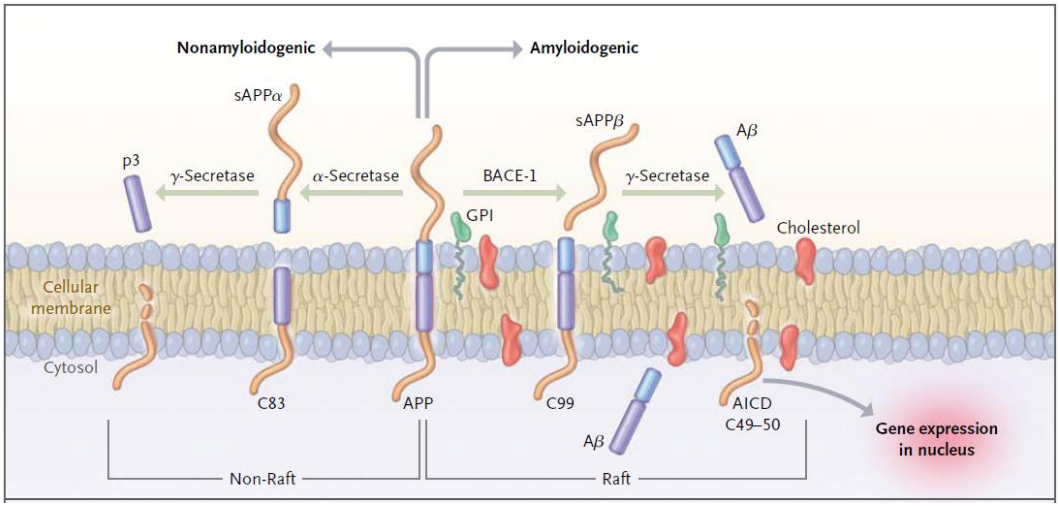

Figura 2: Processamento da Proteína Precursora Amilóide. A APP pode ser clivada por duas vias. A maior parte ocorre através da não-amiloidogênica, que evita a formação de Aβ (à esquerda) por consistir na clivagem dentro do domínio do peptídeo. A primeira clivagem enzimática é mediada pela α-secretase e resulta na liberação de dois fragmentos, um ectodomínio comprido (sAPPα) e um fragmento carboxiterminal (C83). Este pode, posteriormente, ser clivado pela y-secretase, formando um resíduo p3 e um domínio intracelular amilóide (AICD). A via amiloidogênica (à direita) é iniciada pela clivagem pela β-secretase, liberando um resíduo mais curto (sAPPβ) e deixando um fragmento C99. Este também é clivado pela y-secretase, resultando na produção de Aβ e AICD, que é translocado para o núcleo e age como um fator de transcrição. (Retirado de Querfurth and LaFerla, 2010) [7]

Os emaranhados neurofibrilares são estruturas formadas intracelularmente que ocorrem tanto na DA quanto em outras desordens neurodegenerativas, as chamadas tauopatias. O seu principal componente é a proteína tau, que se encontra anormalmente fosforilada e agregada. Em situações normais, a tau é uma proteína solúvel abundante no axônio e promove a estabilidade dos microtúbulos e transporte vesicular. Em estado super fosforilado, porém, ela se torna insolúvel, perde afinidade pelos microtúbulos e junta-se formando inclusões [8]. Estas são citotóxicas e podem causam danos cognitivos [1].

1.3. Patogênese

Apesar da etiologia ainda desconhecida da doença, propõe-se que a deposição do peptídeo Aβ seja o evento inicial do processo, que leva à formação das placas senis e dos emaranhados neurofibrilares (hipótese da cascata amilóide) [9]. Alguns mecanismos relacionados com a patogênese da doença são descritos a seguir (revisados por Querfurth e LaFerla, 2010) [7]:

- *Falha sináptica*: os oligômeros de Aβ afetam a plasticidade sináptica através da redução do número de espinhos dendríticos e da capacidade de gerar potenciais de longa duração (LTP), processo importante para a formação da memória. Os Aβ facilitam a endocitose dos receptores de NMDAr e AMPAr e ligam-se aos receptores neurotrofina p75 (p75NTr) e BDNF. Ainda, os oligômeros reduzem a liberação de acetilcolina dos terminais presinápticos;

- *Disfunção mitocondrial*: os oligômeros de Aβ inibem importantes enzimas mitocondriais no cérebro, como a citocromo *c* oxidase, por exemplo. Consequentemente, o transporte de elétrons, a produção de ATP, o consumo de oxigênio e o potencial de membrana sofrem prejuízos. Além disso, a disfunção mitocondrial causa estresse oxidativo, liberação do citocromo *c* e apoptose;

- *Redução dos componentes da via de sinalização de insulina*: levam a déficits energéticos, fazendo com que os neurônios tornem-se vulneráveis

à oxidação e outros insultos metabólicos e reduzindo a plasticidade sináptica. Além disso, concentrações elevadas de glicose sérica causam danos ao hipocampo;

- *Processo inflamatório: na* doença, ocorre aumento da atividade das micróglias, células imunes do sistema nervoso, que internalizam e degradam os Aβ. Porém, quando ativadas cronicamente, essas células liberam quimiocinas e uma série de citocinas citotóxicas, como interleucina-1, interleucina-6 e fator de necrose tumoral α (TNF-α). O processo inflamatório provoca mudanças nos neuritos e pode levar ao rompimento vascular da barreira hematoencefálica (AD);

- *Perda do balanço de cálcio* – leva ao aumento das concentrações citosólicas de cálcio, estimulando a agregação de Aβ, que, por sua vez, forma canais voltagem-dependentes nas membranas lipídicas, resultando na captação de mais cálcio e degeneração de neuritos;

- *Defeitos no transporte axonal*: levam à deposição de Aβ e ao acúmulo de APP, vesículas sinápticas, kinesinas e outros componentes. Esses problemas devem-se, majoritariamente, à função alterada da proteína tau.

1.4. Fatores Genéticos

Com base na idade em que os sintomas clínicos têm início, a doença de Alzheimer pode ser classificada em dois tipos: *early onset Alzheimer's disease* (EOAD) ou Alzheimer familiar e *late onset Alzheimer's disease* (LOAD) ou Alzheimer esporádico. A forma familiar, responsável por 1-5% de todos os casos da doença, acomete indivíduos com menos de 65 anos e é geralmente associada a uma rápida progressão e mutações genéticas de padrão autossômico dominante [10]. A forma esporádica apresenta uma etiologia multifatorial, em que determinados polimorfismos estão envolvidos.

Mutações em três genes têm sido fortemente associadas à forma familiar da DP: *APP*, *PSEN1* e *PSEN2*. Tais mutações levam ao aumento dos níveis do peptídeo Aβ42, sua agregação e o início precoce da doença, que ocorre na quarta ou quinta década de vida. Em contraste, os genes envolvidos no Alzheimer esporádico, considerados genes de

susceptibilidade, aumentam o risco da patologia em um padrão não-Mendeliano [10]. O fator mais fortemente relacionado à LOAD é o alelo APOEε4. A APOE é uma proteína da classe das apolipoproteínas que transporta lipoproteínas, vitaminas e colesterol pela circulação. Ela é expressa em humanos em três diferentes isoformas, APOEε2, ε3 e ε4, sendo ε3 considerado um alelo neutro, ε4 um alelo de alto risco e ε2 um alelo protetor contra a doença de Alzheimer [11]. Recentemente, estudos de *genome-wide association* (GWA) identificaram variantes de outros genes, como *CLU*, *PICALM*, *CR1* e *BIN1*, como possíveis genes de susceptibilidade [12-14].

2. Doença de Parkinson

2.1. Epidemiologia

A Doença de Parkinson (DP), segunda doença neurodegenerativa mais comum atualmente, foi descrita pela primeira vez em 1817, por um médico inglês chamado James Parkinson. Na monografia publicada, intitulada "*An Essay on the Shaking Palsy*" ("Um Ensaio sobre a Paralisia Agitante"), ele descreveu os principais sintomas clínicos da doença e fez considerações a respeito de sua etiologia e tratamento. A DP é uma desordem progressiva em que os sintomas iniciam, na maioria dos casos, por volta 55 anos de idade, e cuja incidência aumenta significativamente com a idade, de 20 em 100.000 habitantes na população em geral para 120 em 100.000 em pessoas com mais de 70 anos [15]. A enfermidade é caracterizada pela morte de neurônios dopaminérgicos na via *nigro-estriatal* e tem como principais sintomas bradicinesia, hipocinesia, rigidez, tremor de repouso e instabilidade postural. Além dos distúrbios motores, pacientes com a doença podem apresentar déficits cognitivos, como demência, depressão, psicose, confusão e distúrbios de sono [16].

Embora quase dois séculos tenham passado desde sua descoberta, os mecanismos moleculares envolvidos na patogênese da DP ainda não foram elucidados e não existem fármacos capazes de retardar ou impedir a

neurodegeneração característica da doença. Apesar do desenvolvimento de Levodopa (fármaco de escolha para síndromes parkinsonianas) ter revolucionado o tratamento da DP, seu efeito é paliativo e seu uso prolongado apresenta altos riscos de desenvolvimento de discinesias graves e incapacitantes [17]. O processo de neurodegeneração tem início aproximadamente uma década antes das primeiras manifestações clínicas, de modo que, no momento do diagnóstico, estima-se que já haja morte de cerca de 50-70% dos neurônios da *substância nigra* [18], o que representa uma grande limitação para o tratamento da DP.

A ideia de que a DP pode ser causada por fatores ambientais surgiu com a observação, em 1983, que a injeção intravenosa de drogas contaminadas com 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) causava os sintomas típicos da doença [19]. Outros fatores, tais como realização de atividades físicas, exposição a pesticidas, trauma, depressão, distúrbios olfatórios e ansiedade estão sendo associados a um aumento no risco do desenvolvimento da DP [15].

2.2. Características Neuropatológicas

A Doença de Parkinson tem como principais características a morte dos neurônios dopaminérgicos da via *nigro-estriatal* e a presença dos Corpos de Lewy (LB) (**Figura 3**). Os LB são inclusões protéicas compostas primariamente por alfa-sinucleína, parkina, ubiquitina e neurofilamentos, e são encontradas em todas as áreas do cérebro afetadas pela doença [16].

Os corpos celulares dos neurônios da *substância nigra pars compacta* (SNpc) projetam-se primariamente para o putâmen. A perda dessas células, que contém grande quantidade de melanina [20], leva a uma clássica característica neuropatológica da DP: a despigmentação da SNpc (**Figura 4B**). Os neurônios dopaminérgicos mesolímbicos, cujos corpos celulares residem na área tegmentar ventral (VTA), são muito menos afetados na doença. Consequentemente, há significativamente menos depleção de DA no caudado, para onde eles se projetam (**Figura 4A**) [21].

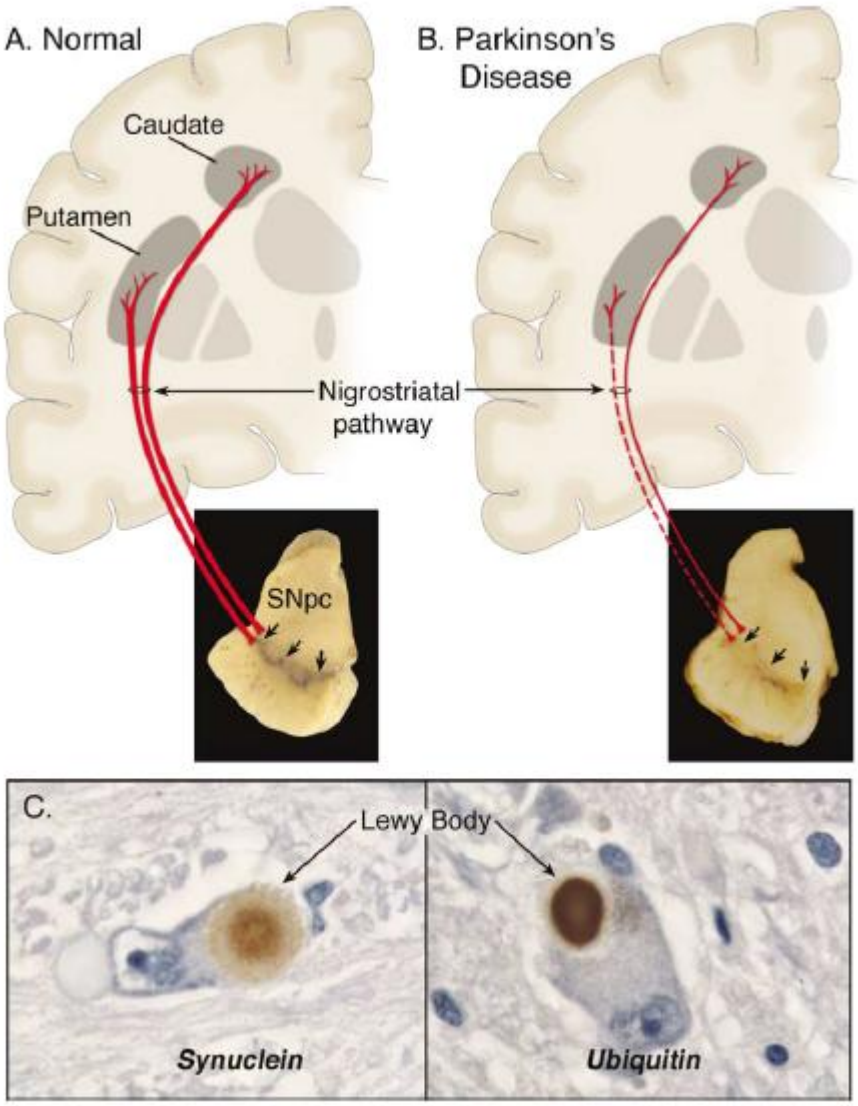

Figura 3: Neuropatologia da Doença de Parkinson. (A) Uma representação esquemática da via *nigro-estriatal* (em vermelho) em situação normal, mostrando os neurônios dopaminérgicos cujos corpos celulares se localizam na substancia nigra e os corpos se projetam para o estriado. Na doença de Parkinson, (B) há despigmentação da substancia nigra em virtude da morte desses neurônios dopaminérgicos (linhas pontilhadas), especialmente aqueles que se projetam para o putâmen. (C) Marcação imunicitoquímica dos Corpos de Lewi, mostrando as proteínas sinucleína e ubiquitina, importantes componentes dessas inclusões. (Retirado de Dauer e Przedborski, 2003) [15]

2.3. Patogênese

A patogênese da doença vem sendo atribuída a dois principais mecanismos distintos: (1) dobramento anormal e agregação protéica e (2) disfunção mitocondrial e estresse oxidativo, que podem ocorrer mutualmente e interagir, conforme proposto na **Figura 4**.

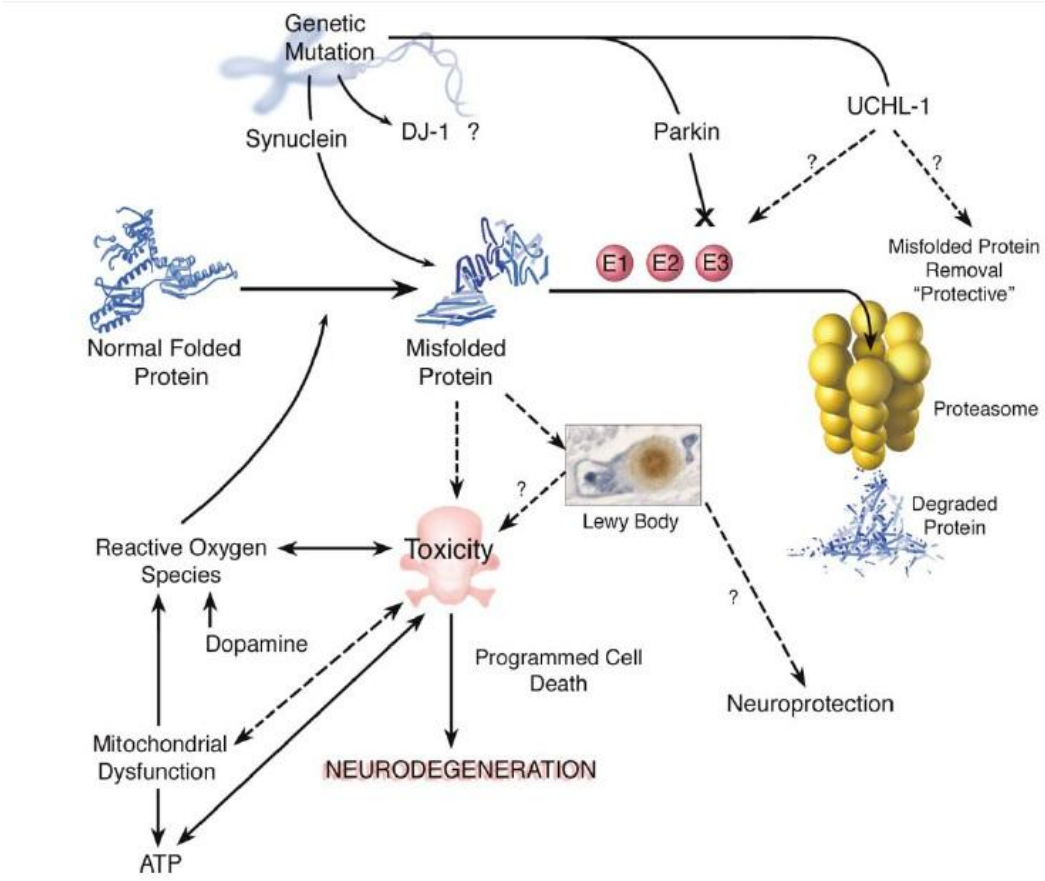

Figura 4: Mecanismos patogênicos envolvidos na Doença de Parkinson. Evidências apontam para que o dobramento anormal de proteínas é um evento central na doença. Mutações patogênicas podem induzir diretamente a mudança da conformação protéica (como se acredita ser o caso da α-sinucleína) ou danificar a maquinaria celular responsável por detectar e degradar proteínas defeituosas, como a via ubiquitina-proteassoma (Parkina, UCH-L1). O papel da DJ-1 ainda precisa ser elucidado. O estresse oxidativo, associado à disfunção mitocondrial e metabolismo anormal de dopamina, também pode gerar proteínas com conformações alteradas. (Retirado de Dauer e Przedborski, 2003) [15]

- *Dobramento anormal e agregação protéica* - a maior parte das mutações responsáveis pela DP familiar resultam na formação de proteínas com conformação anormal ou interferem indiretamente nesse processo por causar falhas nos mecanismos celulares de reconhecimento e processamento de proteínas com dobramento incorreto. Mecanismos relacionados com a alteração do metabolismo de proteínas disfuncionais também vêm sendo considerados como sendo as principais causas do Parkinson esporádico, através de fatores que induzem modificações

estruturais nas proteínas ou na via ubiquitina-proteassoma, como o estresse oxidativo e herbicidas [22].

Além disso, a capacidade da célula de lidar com proteínas anormais parece ser dependente da idade, uma vez que tanto a atividade das chaperonas quanto do proteassoma parecem estar reduzidas em indivíduos idosos. Desse modo, fatores implicados na patogênese da doença, como idade e estresse oxidativo, podem convergir para formar um insulto proteotóxico à célula [23].

- *Disfunção mitocondrial e estresse oxidativo - a* descoberta de que o MPTP, composto que dá origem aos mesmos sintomas que a DP, por causar a morte de neurônios a partir do bloqueio do complexo I da cadeia respiratória da mitocôndria, levou à ideia de que defeitos na fosforilação oxidativa podem estar associados à patogênese da DP [24]. Estudos subsequentes deram força a essa hipótese ao identificar anormalidades na atividade do complexo I em pacientes com DP, que levam ao estresse oxidativo e falhas energéticas [25].

Além de apresentar marcadores biológicos de dano oxidativo elevado, a SNpc de indivíduos com a doença também apresenta conteúdo reduzido de glutationa, um importante anti-oxidante do sistema nervoso [26]. Além disso, o metabolismo da dopamina tem como consequência a formação de peróxido de hidrogênio e radical superóxido, e a oxidação da dopamina produz DA-quinona, que pode reagir com resíduos de cisteína. As falhas energéticas relacionadas a danos mitocondriais podem prejudicar o armazenamento vesicular de DA e causar o extravasamento da mesma no citosol [27]. Assim, os neurônios dopaminérgicos, mais afetados na DP, representam um ambiente particularmente fértil para a formação de espécies reativas de oxigênio (ROS), suportando a ideia de que o estresse oxidativo está intimamente relacionado com a doença.

2.4. Fatores Genéticos

Assim como na DA, as causas da Doença de Parkinson não são claras e acredita-se que mutações gênicas sejam responsáveis por apenas uma pequena proporção dos casos (cerca de 10%). Pouco mais de dez genes vêm sendo associados à doença de Parkinson, entre os quais pode-se citar α-synuclein (*SNCA*), ubiquitin C-terminal hydrolase like 1 (*UCH-L1*), parkina (*PRKN*), *LRRK2*, *PINK1* e *DJ-1*. Apesar de esses genes serem responsáveis apenas por uma pequena parcela dos casos da doença, o estudo das respectivas proteínas codificadas por eles vem auxiliando na elucidação dos mecanismos por trás da patogênese da DP [28].

Além disso, diversos estudos de associação estão sendo realizados para identificar genes que contribuam para o desenvolvimento do Parkinson tipo esporádico, uma vez que se acredita que este dependa de uma interação entre fatores ambientais e genéticos. Apenas alguns polimorfismos (nos genes *NAT2*, *MAOB*, *GSTT1*, alelo ε2 da *APOE*, por exemplo) parecem estar associados à DP [28]. Porém, a suposta multifatoriedade envolvida na doença faz com que o estudo desses genes precise ser realizado e analisado com cautela. Além do efeito individual de cada gene poder ser pequeno e de difícil detecção, certos polimorfismos podem representar um fator de risco para a doença apenas quando associados a determinadas condições ambientais [29].

III. Biologia de Sistemas e Neurociências

Em virtude da imensa quantidade de informações geradas atualmente nas mais diversas áreas do conhecimento, a ciência moderna caracteriza-se por sua crescente especialização. Assim, grandes áreas de estudo são divididas em disciplinas que, por sua vez, estão continuamente gerando novas subdisciplinas [30]. Essa abordagem, conhecida como reducionista, quando aplicada às ciências naturais, consiste na segregação de sistemas biológicos em suas partes constituintes, para que estas possam ser estudadas aprofundadamente, através da utilização de técnicas adequadas

e conhecimento teórico específico. Porém, os sistemas biológicos são extremamente complexos e possuem propriedades emergentes que não podem ser explicadas ou preditas pelo estudo de suas partes de forma individual [31].

A biologia de sistemas é um novo ramo da biologia que surgiu como resultado da necessidade de integrar diferentes áreas para possibilitar uma visão mais ampla dos processos biológicos. Ela tem como foco a compreensão não apenas dos componentes de um sistema individualmente, mas também das interações entre eles e a relação do sistema com o ambiente em que se encontra. Para tanto, a biologia de sistemas emprega métodos de genômica e proteômica integrados a ferramentas desenvolvidas na física e matemática, tais como dinâmica não-linear, teoria de controle e modelagem da dinâmica de sistema, para elucidar processos fisiológicos [32]. Além disso, diferentemente da visão reducionista, em que o estudo se dá com o objetivo de testar uma hipótese prévia (*hypothesis driven*), a biologia de sistema tem como um de suas características a realização e análise de experimentos utilizando uma filosofia holística sobre a funcionalidade e a complexidade dos sistemas biológicos (*fishing experiments*) [33]. As principais diferenças entre as abordagens da biologia clássica e biologia de sistemas estão ilustradas na **Figura 6**:

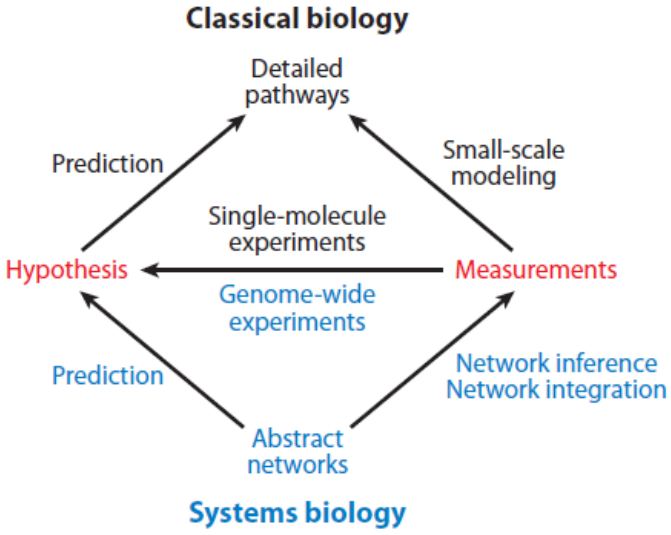

Figura 6: Visão geral dos processos experimentais na biologia clássica (acima) e na biologia de sistemas (abaixo). (Retirado de Chuang et al., 2010) [33]

Dados provenientes dos estudos ômicos são disponíveis em plataformas públicas ou privadas para que possam ser continuamente analisados a partir de diferentes abordagens e metodologias. Assim, o mesmo estudo pode trazer resultados que vão além daqueles encontrados no trabalho original. O *Gene Expression Omnibus* (GEO; http://www.ncbi.nlm.nih.gov/geo/), mantido pelo "US National Institutes of Health", é o maior repositório de acesso aberto disponível, onde são armazenados e disponibilizados milhares de bancos de dados de microarranjo. Através da comparação entre diferentes estudos disponíveis nesse repositório, é possível avaliar diversos componentes de sistemas biológicos e suas alterações quando submetidos a diferentes condições, o que representa o alicerce sob o qual se iniciam as análises de biologia de sistemas. A partir de dados de expressão gênica, existem muitas ferramentas que permitem modelar redes de interação entre os diversos elementos estudados, conferindo uma visão ampla e dinâmica dos componentes do organismo [34]. Um método envolvendo esta abordagem, por exemplo, é a identificação de fatores de transcrição (TFs) que se encontram ativados ou inibidos em condições específicas, os chamados reguladores mestres (MRs). No contexto da neuropatogênese, redes regulatórias específicas das doenças, inferidas a partir de algoritmos de engenharia reversa, podem prover acurácia suficiente para estimar a atividade de fatores de transcrição a partir de seus alvos transcricionais (*regulon*) e identificar aqueles que são os reguladores mestres da doença [35].

Apesar dos grandes avanços na compreensão de processos celulares e moleculares envolvidos nas atividades neuronais, um dos maiores desafios em neurociência atualmente é desvendar a maneira através da qual circuitos cerebrais agem em nível sistêmico e a forma como interagem para dar origem a propriedades complexas como memória, aprendizado e comportamento, por exemplo [30]. A heterogeneidade celular do sistema nervoso, a complexidade dos circuitos neurais e a escassez de material *post-mortem* humano para ser estudado são algumas razões que fazem com que métodos genômicos de triagem em *high-throughput* venham sendo

adotados mais lentamente em neurociência quando comparado a outras áreas biológicas. Apesar disso, essa abordagem sistêmica já se mostrou eficiente para a identificação das bases moleculares da diversidade neuronal, sinaptogênese, vias metabólicas, biomarcadores e mecanismos de doenças [32].

Uma área de estudo intensamente investigada em neurociências é o desenvolvimento de terapias que não apenas tratem os sintomas das enfermidades que acometem o cérebro, mas que também modifiquem a patogênese da doença para preveni-la ou eliminá-la. Para que essa meta seja alcançada, muito da dinâmica das sinapses e circuitos neuronais ainda precisa ser elucidado [32]. A identificação de mutações gênicas associadas com uma doença é, muitas vezes, seguida da tentativa de desenvolver terapias que tem como o objetivo corrigir a função alterada. Porém, a heterogeneidade genética e a falta de conhecimento acerca da contribuição relativa de cada elemento para o desenvolvimento da doença vem sendo responsável pelo fracasso de muitos estudos. Ainda, processos como mielinização, neuroinflamação, insultos isquêmicos e envelhecimento também estão comumente envolvidos na patogênese de doenças neurodegenerativas e precisam ser analisados em conjunto. A abordagem holística da biologia de sistemas tem o potencial de integrar esses diferentes elementos, conectar vias moleculares a funções do sistema nervoso e, deste modo, auxiliar e acelerar o processo de desenvolvimento de fármacos e reduzir seus custos [34].

IV. Artigo Científico

O trabalho a seguir foi formatado de acordo com as instruções para submissão de artigos da revista *Brain Research* (Anexo)

Identification of Transcription Factors that Act as Master Regulators in Alzheimer's Disease and Parkinson's Disease

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Abstract

Alzheimer's Disease (AD) and Parkinson's Disease (PD) are the two most common neurodegenerative disorders. It is estimated that more than 45 million people worldwide suffer from one of these pathologies. Despite the large investment in the neuroscience field, etiology and molecular

mechanisms underlying neuronal death remain unclear. Recently, a small proportion of cases of AD and PD have been attributed to mutations in specific genes. However, the many pathways in which their gene products are involved and the interaction with other factors that might lead to neuropathological changes are still poorly understood. In the current work, microarray data acquired from the public platform Gene Expression Omnibus (GEO) (GSE60862) was used to determine normal tissue-specific transcriptional networks for hippocampus and *substantia nigra*, structures specifically damaged in AD and PD, respectively. Case-control studies (GSE5281 and GSE8397) were used to establish gene expression signatures for the two neurodegenerative diseases and identify transcription factors (TFs) that are pivotal to modulate phenotypic changes from normal to pathological contexts, called master regulators (MRs), applying MRA and GSEA. As results, we identified 117 important TFs regulating gene expression in hippocampus and 123 in *substantia nigra*. We proposed 17 MRs involved with AD and 28 with PD, some of which have already been described in the process of neurodegeneration (such as *YY1*, *HMG20A*, *RREB-1* and *SLC3A9*) and others not related to the pathologies so far. We believe that these results might help in the understanding of AD and PD and lead to the discovery of targets for potential therapeutic intervention.

Keywords: Alzheimer's Disease, Parkinson's Disease, transcription factor, master regulator, gene signature.

1. Introduction

Neurodegenerative disorders represent one of the greatest healthcare challenges faced by modern society. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most prevalent neurodegenerative pathologies. They affect millions of people worldwide and generate huge economic and human costs. These disorders are incurable, progressive conditions that result from continuous degeneration of nerve cells, leading

invariably to death within a few years (Skovronsky et al., 2006). Once the prevalence and incidence of neurodegenerative diseases increase dramatically with age, the number of individuals affected by these disorders is expected to augment even more in the future as the life expectancy continues to grow (Checkoway et al., 2011). Despite of countless studies being carried out and unquestionable advances reached in the last decades in the neurosciences field, the etiology and molecular mechanisms underlying the pathogenesis of these diseases are still poorly understood. The identification neurodegenerative disorders is based on imaging methods or clinical evaluation of the symptoms, which are generally manifested after the irreversible loss of a great extent of neurons. Moreover, available therapy is essentially palliative and incapable of halting or preventing the degenerative process (Skovronsky et al., 2006).

Alzheimer's disease is the most common neurodegenerative disease. More than 35 million people worldwide suffer with the pathology, which is characterized by the deterioration of memory and other cognitive domains. The pathological hallmarks of AD include the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles accompanied by reactive microgliosis, dystrophic neurites, and loss of neurons and synapses (Serrano-Pozo et al., 2011). The initiating insult of the disease is not clear, but it is believed to arise from alterations in the production and processing of amyloid β (Reitz, 2012). Parkinson's disease results mainly from the death of dopaminergic neurons in the *substantia nigra pars compacta* and is characterized by the presence of intracellular inclusions known as *Lewy Bodies* (LB). Symptoms include resting tremor, rigidity, bradykinesia, postural instability and cognitive deficits (Davie, 2008).

The causes of both PD and AD are still largely unknown. Even though a small proportion of cases is driven by genetic mutations in specific genes, the great majority of them (>90%) is considered to be sporadic and idiopathic, induced by non-genetic factors probably in interaction with susceptibility genes (Dauer and Przedborski, 2003). Therefore, the study of isolated gene mutations related with these neuropathologies might underestimate the complexity of the process of neurodegeneration. However,

moving from single gene approaches to an understanding of interacting signaling or metabolic pathways within cells, and further combination of these data to achieve a systems-level comprehension of brain circuit function in health and disease, represent one of the greatest challenges in the field of neuroscience (Geschwind and Konopka, 2009).

In this context, system biology arises as an option to improve our ability to characterize organisms and processes systemically. It is based on the idea that biological systems are extremely complex and have emergent properties that cannot be explained, or even predicted, by studying their individual parts (Van Regenmortel, 2004). Therefore, system biology approach relies on large-scale techniques, such as omic tools (genomic, transcriptomic, proteomic and metabolomic). The adoption of functional genomic or molecular systems methods that permit dynamic measurement of elements in different hierarchical levels of the organism in a highly parallel manner has the potential to provide a more integrative understanding of nervous system function (Geschwind and Konopka, 2009). One of these methods is the high-throughput microarray *in situ* hybridization, which displays messenger RNA expression in a given section of brain and provides valuable information regarding the spatial and temporal expression of every gene in the nervous system under a certain condition. This distinct pattern of gene expression throughout the brain, which grants different neuronal features and tissue-specific functions, is mainly regulated by transcription factors (TFs) (Qian et al., 2005). Therefore, the study of these components provides insights into the molecular basis of central nervous system development and processes that give rise to higher cognitive complexity. Moreover, TFs' ability to regulate targets is modulated by a variety of genetic and epigenetic mechanisms, resulting in highly context dependent regulatory networks (Bansal and Califano, 2012). Thus, the use of microarray transcriptomic data enables the identification of TFs that are pivotal to modulate phenotypic changes from normal to pathological contexts, the called master regulators (MRs) for the condition (Carro et al., 2010).

In neurodegenerative diseases, the analysis of MRs might contribute to the understanding of these pathologies and the discovery of targets for

potential therapeutic intervention. Hence, our primary goals are to identify tissue-specific transcriptional regulatory networks of hippocampus and *substantia nigra* of healthy individuals and propose MRs responsible for coordinating expression of genes that result in the pathological phenotype in both Alzheimer's disease and Parkinson's disease.

2. Results

Clinical manifestations of Alzheimer's and Parkinson's diseases are mainly attributed to the significant neuronal loss in hippocampus and *substantia nigra,* respectively. In order to further understand the normal and pathological states of these structures, we modeled tissue-specific transcriptional networks for them, using mRNA expression data of both tissues from *post-mortem* patients obtained from large-scale studies available on GEO Consortium (GSE60862) and a list of TFs available on Gene Ontology Consortium (GO:0003700).

The microarray data was pre-processed and probes with low variation removed from the analysis. The relation between every TF and the group of genes it modulates (its regulon) was established by computing the mutual information (MI) between annotated transcription factors (TFs) and all potential targets in the dataset. In this reference network each target could be linked to multiple TFs and regulation could occur as a result of both direct (TF – target) and indirect interactions (TF – TF – target). In order to preserve the dominant TF – target pairs for the subsequent enrichment analyses, we additionally applied the Data Processing Inequality (DPI) algorithm, which removes the weakest interaction in any eventual triplet formed by two TFs and a common target gene. Regulatory units with more than 100 targets were considered relevant to the structure.

Out of 821 TFs considered (details in **Supplementary Table 1**), our initial analysis revealed that 117 seem to be important for regulation of gene expression in hippocampus and 123 in *substantia nigra* (**Figures 1 and 2**). Moreover, both structures present tissue-specific and common TFs. Venn diagram summarizing these results is shown in **Figure 3**.

Next, the normal transcriptional network models inferred for each structure were used to identify regulatory units for our diseases of interest. For that, we performed differential expression analysis comparing mRNA expression from AD or PD patients and controls, using data obtained from two microarray studies (GSE5281 for AD and GSE8397 for PD). For the first dataset, samples were obtained from *post-mortem* brains of male and female individuals between 63 and 102 years diagnosed or not with Alzheimer's disease. Microdissection capture on 6 brain regions was carried out and layer III pyramidal cells from the white matter in each region were collected. In the second study, *post-mortem* brain tissue samples from *substantia nigra*, split into medial and lateral portions, and frontal cortex, were obtained from 47 Parkinson's disease and control cases, of both genders, ranging from 46 to 89 years.

Two different analyses, Master Regulator Analysis (MRA) and Gene Set Enrichment Analysis (GSEA), were used to test the expression differences in regulons, thus identifying the MRs for the conditions. For PD, 71 TFs were identified as master regulators with MRA and 28 with GSEA. For AD, 89 MRs were suggested by MRA and 18 by GSEA. (**Figures 4** and **5**, **Supplementary tables 2-5**). Common TFs inferred by both tests were considered relevant master regulators to the diseases (**Figure 6)**, some of which are specifics for only one condition while others appear to be shared by both of them (illustrated in Venn diagram in **Figure 7**).

3. Discussion

The brain is a highly complex organ, where many unique functions are performed in discrete regions. Characterization of each region and identification of its molecular singularity is critical to the understanding of complexity of higher cognitive functions (Suzuki et al., 2004). Cellular differentiation, histogenesis, and development are the consequences of differential gene expression, which result mainly from the action of different transcription factors. Moreover, TFs are a common way in which cells respond to extracellular information, such as environmental stimuli and signals from other cells (Clevidence et al., 1993). Therefore, the identification

and characterization of region-specific gene regulatory networks might yield insights into the comprehension of the molecular basis of a tissue's development, normal function and pathology.

Our study allowed the identification of transcriptional networks which seem to be responsible for morphological features and cellular activity of hippocampus and *substantia nigra,* shown in **Figures 1** and **2**. The relative contribution of 821 TFs for the molecular signature of both structures was analyzed, out of which 117 seemed to be important in hippocampus and 123 in *substantia nigra*. As expected, due to the cellular heterogeneity and highly specialized functions in each structure, several tissue-specific TFs were found to play a role in either hippocampus or *substantia nigra* (**Figure 3**). Among the 54 TFs identified exclusively in hippocampus, several were consistent with data in previous reports, such as *YY1*, *PPARA*, *NR3C2*, *NFKB1*, *NEUROD2*, *MEF2A* and *EMX1* (Suzuki et al., 2004). Similarly, among TFs found in *substantia nigra* only, is *HIF1A*, reported to be involved in development of dopaminergic neurons (Milosevic et al., 2007), and several TFs related to regulation of circadian rhythm. Interestingly, dysfunction of circadian clock has recently been related to dopaminergic neuron loss in animal models of PD (Tanaka et al., 2012, Willison et al., 2013). This information indicates reliability of our results. Besides specific features of each region, 63 common TFs were present in both of them, probably contributing to general neuronal features and basic cellular processes essential to cellular survivor.

Regarding the determination of regulatory units for Alzheimer's and Parkinson's diseases, common MRs identified by both MRA and GSEA were investigated. **Figures 4** and **5** show the inferred transcriptional networks for hippocampus and *substantia nigra* and the regulons differentially expressed in PD or AD using each of the analyses.

When comparing the MRs identified for the two pathologies, summarized in **Figure 6**, it is possible to notice that most of the TFs proposed are not exclusively related to a single disease (**Figure 7**). This observation probably reflects the many common molecular events that might underlie the process of neurodegeneration.

In fact, only one MR, *YY1*, was identified exclusively in Alzheimer's Disease. This neurodegenerative condition's hallmarks are the presence of amyloid plaques (composed mainly by Aβ) and neurofibrillary tangles (hyperphosphorylated tau). The transcription factor Yin Yang 1 (*YY1*) is a multifunctional protein which possible relation in this disorder has already been reported (He and Casaccia-Bonnefil, 2008). Aβ is proteolytically cleaved from amyloid precursor protein (APP) by β and γ-secretase and BACE1 (beta-site amyloid precursor protein-cleaving enzyme 1) (O'Brien and Wong, 2011). *YY1* acts as an activator of the BACE1 promoter in neurons and astrocytes, thus controlling the amount of Aβ generated. Still, there are evidences that *YY1* might regulate the levels of Aβ indirectly, by modulating the expression of other molecules involved in APP processing, such as FE65 (Nowak et al., 2006, He and Casaccia-Bonnefil, 2008).

Related solely to Parkinson's disease, our analysis suggested a total of 10 MRs, among which some have also been previously reported to possibly play a role in this disorder. Even though the pathogenesis of PD is not clear yet, it is believed that two major mechanisms are responsible for the initiation and progression of the pathology: (1) misfolding and aggregation of proteins (mainly α-synuclein) and (2) mitochondrial dysfunction and oxidative stress (Dauer and Przedborski, 2003). TFs affecting both mechanisms were suggested as master regulators. *HMG20A* has been reported to inhibit α-synuclein fibrillization, process which originates the inclusions called *Lewy Bodies*, an important characteristic of Parkinson's disease. Therefore, changes in *HMG20A* expression might directly contribute to the disorder (Shah, 2013, Lindersson et al., 2004). *ECSIT* (an evolutionary conserved signaling intermediate in Toll pathways) is a key component of the oxidative phosphorylation system. It is required for the assembly of mitochondrial complex I (NADH:ubiquinone oxidoreductase) and its absence implicates in accumulation of intermediates and mitochondrial dysfunction, resulting in the activation of pro-apoptotic mechanisms, synaptic dysfunction and neuronal death (Soler-Lopez et al., 2012). Abnormalities in complex I of the electron transport chain were already described in PD patients (Greenamyre et al., 2001) and underlie the mechanism behind some of the most used toxin-based models of the disease (such as MPTP and rotenone, for example)

(Dauer and Przedborski, 2003). Moreover, *SREBF1* and *RREB-1*, identified as MRs, have also been found to play a role in Parkinson's disease in previous studies (Ivatt et al., 2014; Yamane et al., 2013).

Furthermore, our analysis identified 17 regulons differentially expressed in both Alzheimer's and Parkinson's diseases. Even though neurodegenerative diseases are a group of pathologies characterized by separate etiologies with distinct morphological and pathophysiological features, they are thought to share several common degenerative processes that contribute to neuronal death. Among these, the most important seem to be (a) abnormal protein dynamics with defective protein degradation and aggregation, (b) oxidative stress and free radical formation, (c) impaired bioenergetics and mitochondrial dysfunction, and (d) exposure to metal toxicity and pesticides (Sheikh et al., 2013). In this context, we identified some interesting TF shared by AD which might be involved with the two diseases through one of the mechanisms mention above. *CERS6*, for example, is a TF which mediates apoptosis driven by oxidative stress (White-Gilbertson et al., 2009). Moreover, some MRs, such as *ATF-2* (a member of the activator protein -1 family), are known to provide clinical significance in neurodegenerative diseases, but the way through which they do it is not understood (Pearson et al., 2005; Yamada et al., 1997).

Several MRs identified, such as *ZFP69B*, *CSRNP2*, *PHTF-1*, *HMG20A* and *FOXC2* have been previously linked to the incidence of diabetes, specially type II (*T2D*) (Douroudis et al., 2010; Perry et al., 2012). Interestingly, *T2D*, AD and PD are commonly associated with growing age and *T2D* is now known to be a risk factor for neurodegenerative diseases. The precise mechanism behind *T2D*-related cognitive dysfunction remains to be elucidated, but some hypothesis have been proposed. It is possible that constant high glucose levels *per se* damage cause neuronal damage through osmotic insults and oxidative stress, generating advanced glycation endoproducts (AGEs), which can reactivate microglia in the central nervous system, stimulating the immune response. In addition, studies have demonstrated that insulin signaling in the brain prevents the pathological binding of Aβ oligomeres to synapses, thus the reduction of this molecule

might be also contributing to AD through a specific mechanism (Luchsinger, 2012).

In summary, the present work identified tissue-specific transcriptional regulatory networks of hippocampus and *substantia nigra*, important to the elucidation of both common and specific cellular processes which happen in these structures, contributing to the understanding of molecular basis of development, function and disease of the brain. Moreover, we analyzed these TFs in the context of disease and proposed 18 MRs of AD and 28 MRs of PD. Many of these TFs had already been related to the same pathologies in previous studies, ensuring the reliability of our results. However, further analyses are required in order to make the results more robust. First, other microarray data from case-control studies of PD and AD are needed in order to validate the MRs identified here. Second, it is extremely important to determine whether the regulons regulated by MRs are over- or under-expressed in the context of the disease, applying two-tailed GSEA. Finally, experimental approaches such as chromatin immunoprecipitation are of paramount importance to validate the MRs activity. Moreover, once the MRs of the diseases are established, this data can be used to search not only for drugs that act on these regulatory components but also for compounds capable of producing perturbations that change expression pattern from normal to pathological states. Therefore, we believe that these results, coupled with further analysis, might help in the understanding Parkinson's and Alzheimer's diseases' pathogenesis and etiology and lead to the discovery of targets for potential therapeutic intervention.

4. Experimental Procedures

4.1. Microarray Data

Data used to reconstruct the transcriptional associations in normal human hippocampus and *substantia nigra* were obtained from a large-scale microarray study (GSE60862) extracted from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/). In order to establish diseases

gene expression signatures, independent microarray studies (GSE5281 and GSE8397, for AD and PD, respectively) were used (see **Table 1** for details).

4.2. Transcriptional network inference

The transcriptional networks were constructed using the *R* package *RTN* (Fletcher et al., 2013). Gene probes were filtered based on their coefficient of variation (CV) and mutual information (MI), calculated in the *R* package *minet* (Meyer et al., 2008). The regulatory structure of the network was derived by mapping the significant interactions between known transcription factors and all potential targets in the gene expression matrix. Interactions that were below a minimum MI threshold were eliminated by permutation analysis. Unstable interactions were additionally removed by bootstrap analysis using five thousand bootstrap samples and a *p* value cutoff of 0.001 to create the consensus bootstrap network (*i.e.* the relevance network). In an additional step the Data Processing Inequality (DPI) algorithm was applied with tolerance = 0.0 in order to eliminated interactions that are likely mediated by another TF (Margolin et al., 2006). As the DPI removes the weakest edge of each network triplet, the vast majority of the interactions that were likely to be indirect were eliminated in this step. The resulting DPI-filtered transcriptional network was subsequently interrogated in the enrichment analysis.

4.3. Master regulator and gene set enrichment analysis

The master regulator analysis (Fletcher at al. 2013) and Gene Set Enrichment Analysis (GSEA) were used to assess if a given transcriptional regulatory unit (regulon) was differentially expressed among 2 classes of microarrays. The GSEA uses a rank-based scoring metric obtained from the differentially expressed signatures in order to test the association between gene sets and the ranked phenotypic difference. Here regulons were treated as gene sets and AD or PD signatures as the phenotype, an extension of the GSEA analysis as previously described (Subramanian et al., 2005). The analyses were performed in the *R* package *RTN*. Both MRA and GSEA

considered *P*-value cutoff of 0.05 using Benjamin-Hochberg (BH) adjusting p-value method and a minimum regulon size of 100. The number of permutations used in GSEA was 1000.

4.4. Analysis of gene expression data

The R project Bioconductor package *limma* (Wettenhall and Smyth, 2004) was used to call differentially expressed genes, and the log fold change (logFC) metric was used to obtain the ranked phenotypes required for the GSEA analysis.

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Figure Legends:

Figure 1: Transcriptional network inferred for *substantia nigra*. TFs are shown with their respective regulon, represented by circles. Grey nodes correspond to regulons with between 25 and 99 targets. Red nodes represent regulons with 100 or more targets, considered relevant to the structure and used for further identification of MRs in Parkinson's disease.

Figure 2: Transcriptional network inferred for hippocampus. TFs are shown with their respective regulon, represented by circles. Grey nodes correspond to regulons with between 25 and 99 targets. Red nodes represent regulons with 100 or more targets, considered relevant to the structure and used for further identification of MRs in Alzheimer's disease.

Figure 3 – Venn diagram showing important regulons (>100 targets) identified in hippocampus (red), *substantia nigra* (blue) and both (intersection).

Figure 4 – Master regulators of Parkinson's disease inferred with MRA (pink) and with both MRA and GSEA (red), identified from a tissue-specific transcriptional network previously determined for *substantia nigra.*

Figure 5 – Master regulators of Alzheimer's disease inferred with MRA (pink) and with both MRA and GSEA (red), identified from a tissue-specific transcriptional network previously determined for hippocampus*.*

Figure 6 - Master regulators of Parkinson's Disease (left) and Alzheimer's Disease (right) inferred with both MRA and GSEA. Colors represent different *p-values*.

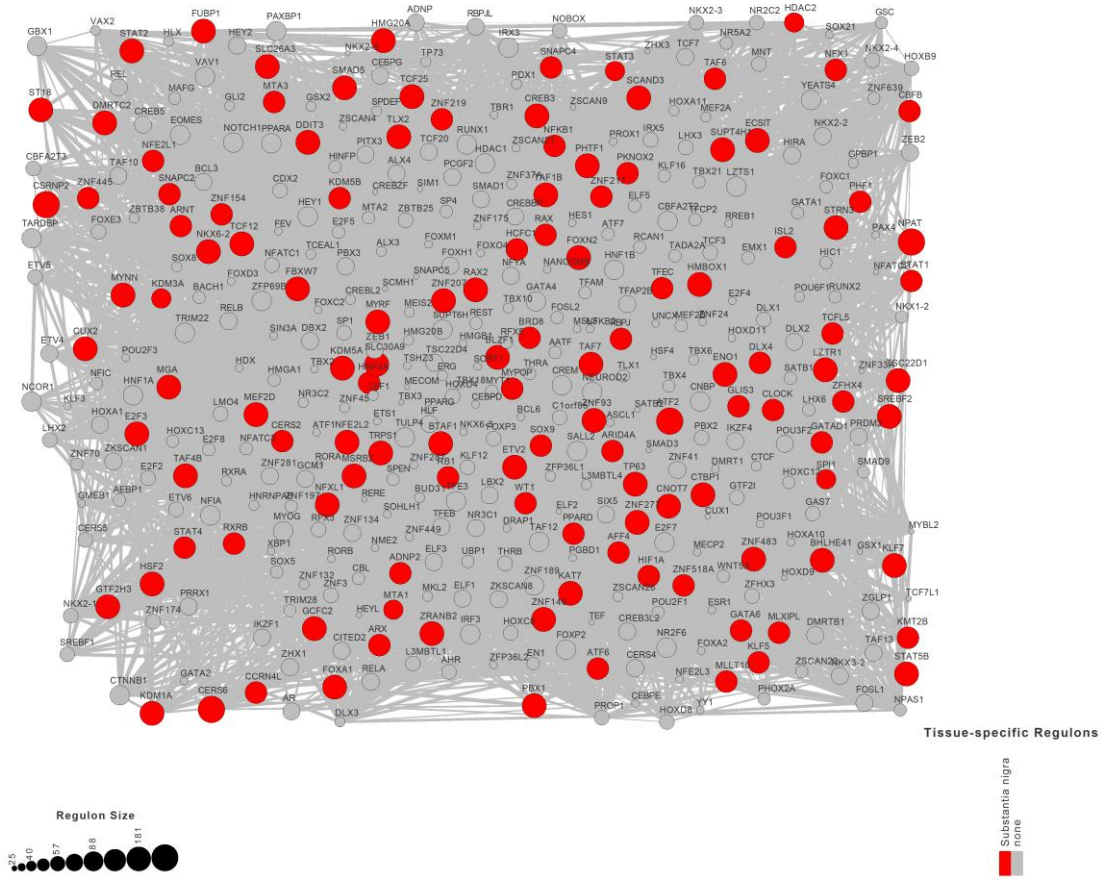
Figure 7: Venn diagram illustrating MRs found exclusively in Alzheimers's disease (red), Parkinson's disease (blue) or shared by the two conditions (intersection).

Table 1

Table 1 - Microarray data used to infer transcriptional networks and identify MRs of AD and PD.

Condition	Series	Description	Samples (n)	Reference
Normal Brain	GSE60862	Gene-level analysis of 1231 samples originating from 134 Caucasian individuals - microarray data from 16 regions of *post-mortem* brains.	Hippocampus (n = 122) / Substantia nigra (n = 101)	Trabzuni et al., *Nat Commun* (2013) 4: 2771.
Alzheimer's Disease	GSE5281	Alzheimer's disease and the normal aged brain - microarray data from 16 regions of *post-mortem* brains.	Healthy subjects (n = 13) / AD patients (n = 10)	Kang et al. *Nature* (2011) 478(7370): 483-9.
Parkinson's disease	GSE8397	Expression profiling of the Parkinsonian Brain - microarray data from 3 regions of *post-mortem* brains.	Healthy subjects (n = 16) / PD patients (n = 24)	Moran et al., *Neurogenetics* (2006) 7(1): 1-11.

Figure 1


Tissue-specific Regulons
Substantia nigra
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Regulon Size
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40
57
88
181

Figure 2

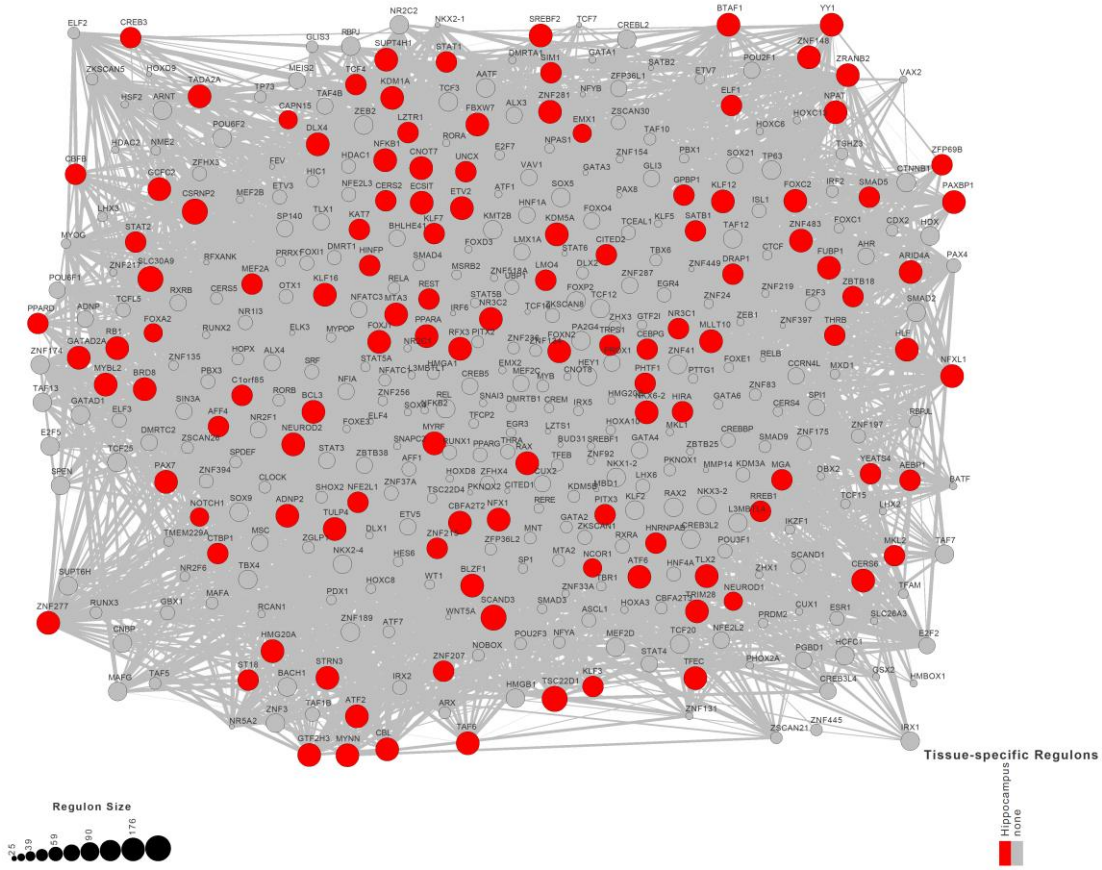

Tissue-specific Regulons
Hippocampus
none
Regulon Size
5
39
59
90
176

Figure 3

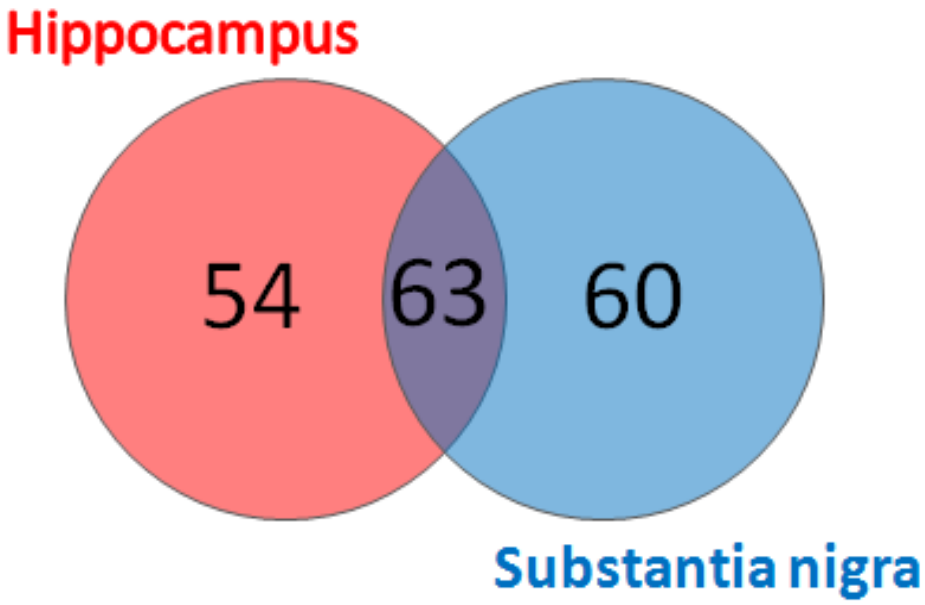
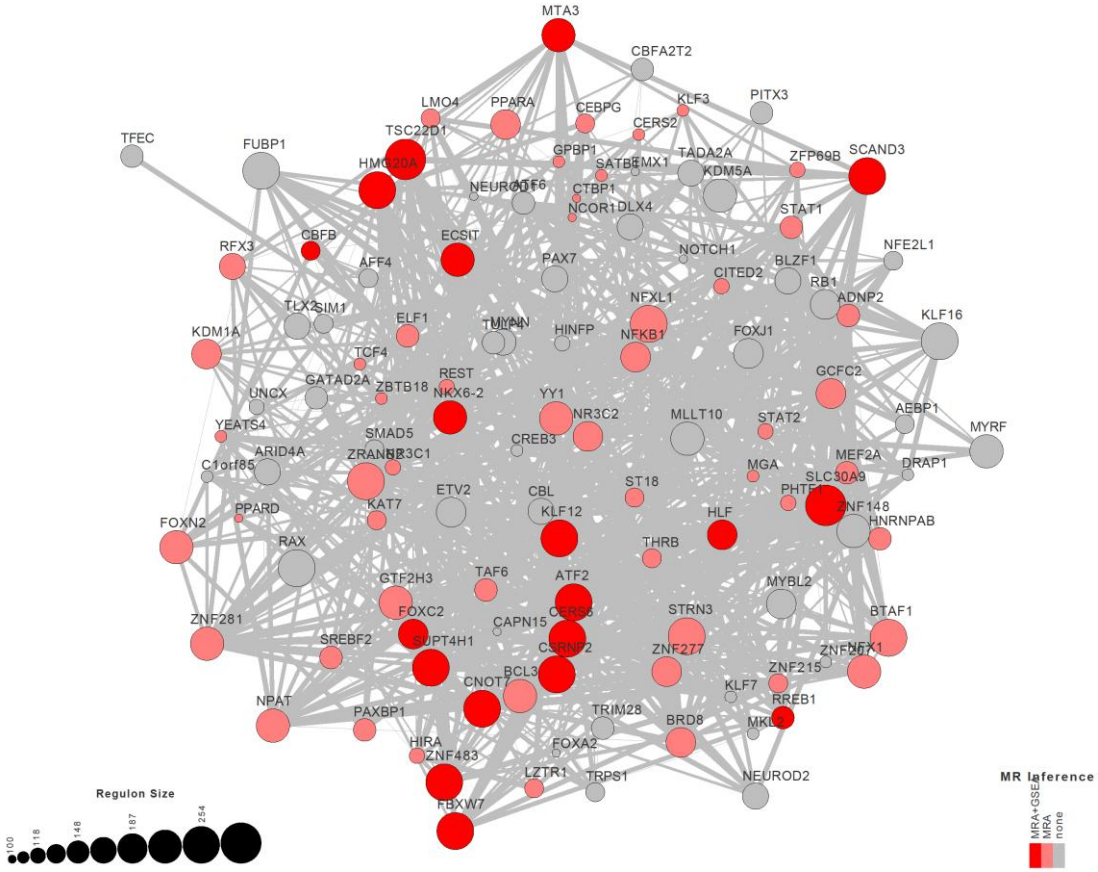


Figure 4


MTA3
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PPARA
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KLF3
PITX3
TFEC
FUBP1
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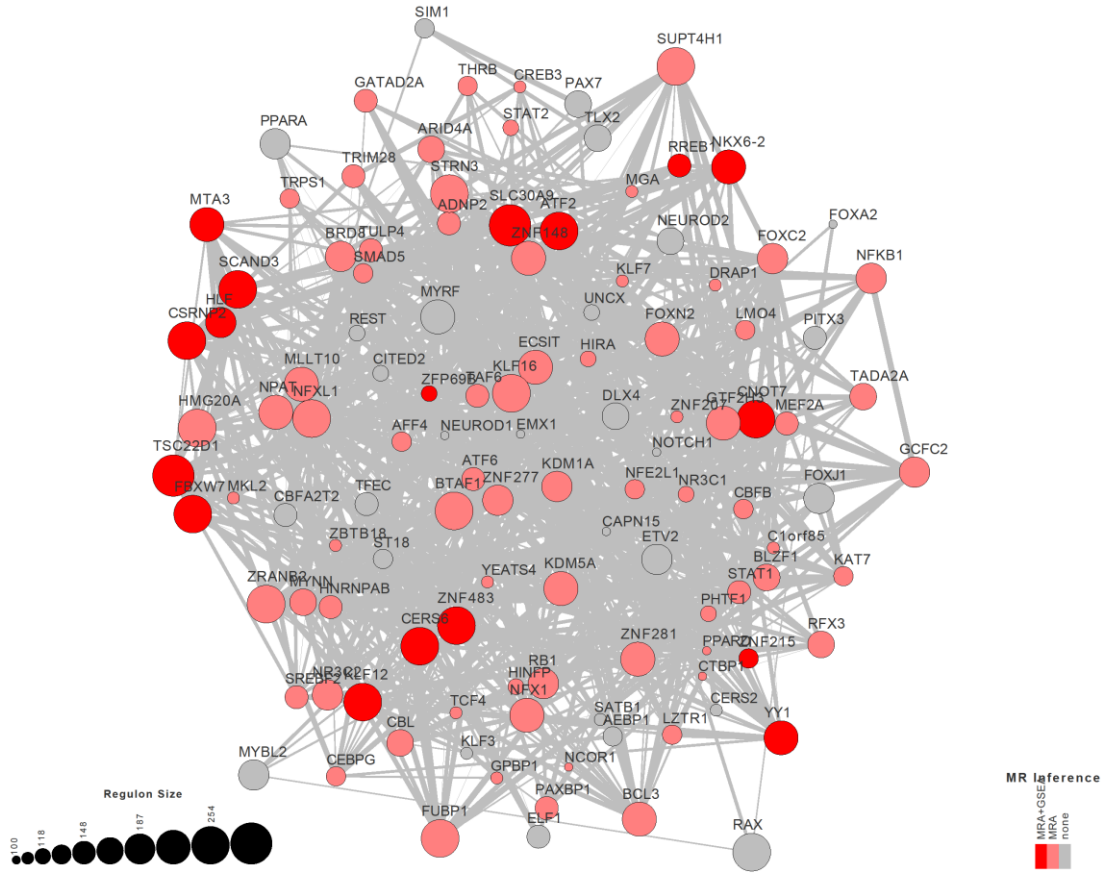
CERS2
GPBP1
TADA2A
ZFP69B
SCAND3
HMG20A
SATB1
MX1
KDM5A
NEUROD6
CTBP1
NCOR1
DLX4
STAT1
RFX3
CBFB
ECSIT
NOTCH1
NFE2L1
AFF4
PAX7
BLZF1
CITED2
RB1
NFXL1
ADNP2
TLX2
SIM1
ELF1
MYAN
HINFP
NFKB1
FOXJ1
KLF16
KDM1A
TCF4
GCFC2
GATAD2A
ZBTB18
REST
NKX6-2
YY1
UNCX
NR3C2
MLLT10
STAT2
AEBP1
MYRF
YEATS4
SMAD5
CREB3
ARID4A
NR3C1
MGA
MEF2A
DRAP1
C1orf85
ETV2
ST18
SLC30A9
PHTF1
PPARD
KAT7
CBL
KLF12
HLF
ZNF148
FOXN2
HNRNPAB
RAX
THRB
GTF2H3
TAF6
ATF2
MYBL2
FOXC2
STRN3
BTAF1
ZNF281
CAPN15
CERS6
SREBF2
SUPT4H1
CSRNP2
ZNF277
ZNF215
BCL3
CNOT7
KLF7
RREB1
NPAT
PAXBP1
TRIM28
BRD8
MKL2
HIRA
ZNF483
FOXA2
LZTR1
TRPS1
NEUROD2
FBXW7
Regulon Size
100
118
148
187
254
MR Inference
MRA+GSE
MRA
none

Figure 5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THRB
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STAT2
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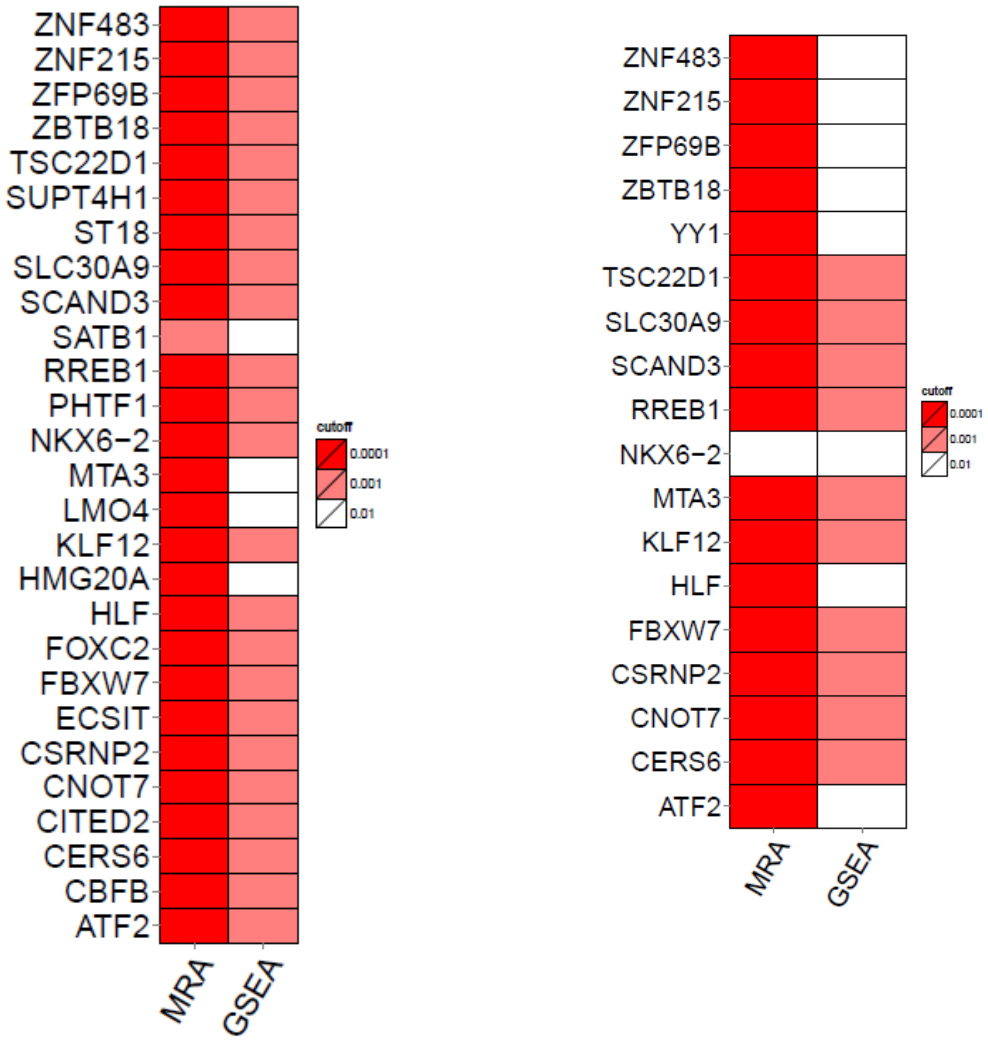
ARID4A
RREB1
NKX6-2
TRIM28
STRN3
TRPS1
MGA
MTA3
ADNP2
SLC30A9
ATF2
FOXA2
NEUROD2
BRD8
ULP4
ZNF148
FOXC2
NFKB1
SCAND3
SMAD5
KLF7
DRAP1
HLF
MYRF
UNCX
CSRNP2
REST
FOXN2
LMO4
PITX3
ECSIT
HIRA
MLLT10
CITED2
KLF16
ZFP69B
TAF6
TADA2A
NPAT
NFXL1
DLX4
CNOT7
HMG20A
ZNF207
MEF2A
AFF4
NEUROD1
EMX1
NOTCH1
TSC22D1
GCFC2
ATF6
KDM1A
NFE2L1
FBXW7
MKL2
BTAF1
ZNF277
NR3C1
FOXJ1
TFEC
CBFA2T2
CBFB
CAPN15
ZBTB18
ETV2
C1orf85
ST18
BLZF1
KAT7
ZRANB2
MYNN
YEATS4
KDM5A
STAT1
HNRNPAB
ZNF483
PHTF1
CERS6
ZNF281
RFX3
PPARD
ZNF215
RB1
CTBP1
SREBF2
NR3C2
REF12
HINFP
NFX1
TCF4
CERS2
SATB1
CBL
AEBP1
LZTR1
YY1
KLF3
MYBL2
CEBPG
NCOR1
GPBP1
PAXBP1
BCL3
FUBP1
ELF1
RAX
Regulon Size
100
118
148
187
254
MR laference
MRA+GSE
MRA
none

Figure 6


ZNF483
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TSC22D1
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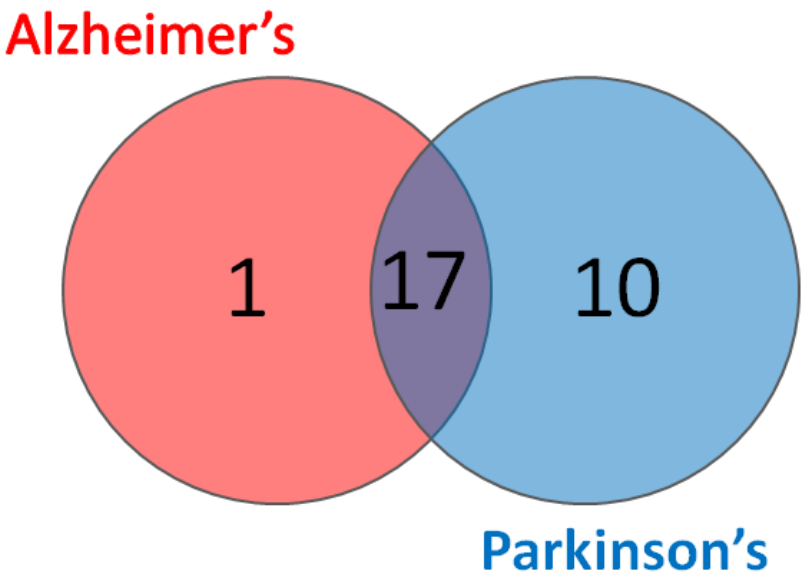
ST18
SLC30A9
SCAND3
SATB1
RREB1
PHTF1
NKX6−2
MTA3
LMO4
KLF12
HMG20A
HLF
FOXC2
FBXW7
ECSIT
CSRNP2
CNOT7
CITED2
CERS6
CBFB
ATF2
MRA
GSEA
cutoff
0.0001
0.001
0.01
ZNF483
ZNF215
ZFP69B
ZBTB18
YY1
TSC22D1
SLC30A9
SCAND3
RREB1
NKX6−2
MTA3
KLF12
HLF
FBXW7
CSRNP2
CNOT7
CERS6
ATF2
MRA
GSEA
cutoff
0.0001
0.001
0.01

Figure 7



Supplementary Table 1 – Tissue-specific transcriptional network inferred from mRNA analysis

Transcription Factor	Regulon size in substantia nigra	Regulon size in hippocampus	Significant TF in substantia nigra	Significant TF in hippocampus	Significant TF in both structures
AATF	50	83			
ADNP	69	75			
ADNP2	110	154	SN	HIP	SN, HIP
AEBP1	45	127		HIP	
AFF1	20	64			
AFF4	137	136	SN	HIP	SN, HIP
AHCTF1	0	5			
AHR	60	68			
ALX1	0	0			
ALX3	37	66			
ALX4	72	70			
ANKRD30A	0	0			
AR	66	16			
ARID3A	10	11			
ARID4A	142	164	SN	HIP	SN, HIP
ARNT	141	92	SN		
ARNT2	0	0			
ARNTL2	0	0			
ARX	114	45	SN		
ASCL1	35	65			
ASCL2	7	6			
ATF1	34	32			
ATF2	507	248	SN	HIP	SN, HIP
ATF3	1	14			
ATF4	0	3			
ATF5	0	0			
ATF6	111	154	SN	HIP	SN, HIP
ATF7	45	52			
ATOH1	0	5			
BACH1	52	97			
BACH2	7	12			
BARX1	19	18			
BARX2	11	17			
BATF	9	30			
BATF2	11	3			
BATF3	0	0			
BCL3	76	213		HIP	
BCL6	36	16			
BHLHE40	11	8			
BHLHE41	165	77	SN		
BLZF1	250	172	SN	HIP	SN, HIP
BNC1	0	4			
BRD8	115	191	SN	HIP	SN, HIP
BSX	18	5			
BTAF1	176	237	SN	HIP	SN, HIP
BUD31	37	26			
C1orf85	64	108		HIP	
CAPN15	14	103		HIP	
CARF	0	0			
CBFA2T2	93	147		HIP	
CBFA2T3	57	52			
CBFB	113	132	SN	HIP	SN, HIP
CBL	48	170		HIP	
CCRN4L	112	78	SN		
CDX2	64	47			
CDX4	9	5			
CEBPA	22	12			
CEBPB	24	18			
CEBPD	29	15			
CEBPE	29	17			
CEBPG	78	126		HIP	

CERS2	116	113	SN	HIP	SN, HIP
CERS3	1	0			
CERS4	80	43			
CERS5	54	53			
CERS6	676	246	SN	HIP	SN, HIP
CIR1	11	21			
CITED1	19	27			
CITED2	66	126		HIP	
CLOCK	132	45	SN		
CNBP	96	93			
CNOT7	239	254	SN	HIP	SN, HIP
CNOT8	2	29			
CREB1	20	23			
CREB3	222	109	SN	HIP	SN, HIP
CREB3L2	79	86			
CREB3L3	5	18			
CREB3L4	1	66			
CREB5	74	67			
CREBBP	71	60			
CREBL2	37	82			
CREBRF	24	NA			
CREBZF	25	13			
CREM	81	37			
CRX	15	18			
CSRNP1	0	4			
CSRNP2	463	410	SN	HIP	SN, HIP
CTBP1	254	104	SN	HIP	SN, HIP
CTCF	39	44			
CTNNB1	92	95			
CUX1	27	28			
CUX2	182	73	SN		
DBX1	5	15			
DBX2	72	33			
DDIT3	215	7	SN		
DLX1	61	28			
DLX2	80	48			
DLX3	39	17			
DLX4	143	165	SN	HIP	SN, HIP
DLX6	18	1			
DMBX1	2	3			
DMRT1	33	64			
DMRT2	14	7			
DMRT3	0	4			
DMRTA1	0	31			
DMRTA2	19	13			
DMRTB1	69	38			
DMRTC2	205	66	SN		
DMTF1	1	4			
DPRX	0	0			
DRAP1	34	106		HIP	
DRGX	14	14			
DUXA	0	0			
E2F1	0	16			
E2F2	55	80			
E2F3	204	62	SN		
E2F4	30	19			
E2F5	46	96			
E2F7	83	42			
E2F8	45	21			
E4F1	0	0			
EBF1	14	12			
ECSIT	277	210	SN	HIP	SN, HIP
EGR1	9	5			
EGR2	2	3			
EGR3	4	38			
EGR4	21	65			
EHF	3	0			
ELF1	73	144		HIP	

ELF2	25	51			
ELF3	65	61			
ELF4	10	26			
ELF5	51	9			
ELK1	3	1			
ELK3	6	25			
ELK4	4	10			
EMX1	46	100		HIP	
EMX2	22	29			
EN1	45	14			
EN2	8	7			
ENO1	344	9	SN		
EOMES	82	23			
ERG	42	20			
ESR1	35	58			
ESR2	3	1			
ESX1	0	0			
ETS1	31	15			
ETS2	16	15			
ETV2	291	193	SN	HIP	SN, HIP
ETV3	23	57			
ETV3L	2	4			
ETV4	75	6			
ETV5	59	68			
ETV6	55	23			
ETV7	0	36			
EVX1	7	16			
EVX2	13	23			
FBXW7	167	281	SN	HIP	SN, HIP
FEV	31	27			
FLI1	4	3			
FOS	9	22			
FOSB	3	2			
FOSL1	75	14			
FOSL2	45	15			
FOXA1	165	11	SN		
FOXA2	35	100		HIP	
FOXA3	6	6			
FOXC1	50	38			
FOXC2	34	180		HIP	
FOXD1	6	3			
FOXD2	6	20			
FOXD3	33	31			
FOXD4	0	0			
FOXE1	22	38			
FOXE3	56	32			
FOXF2	8	3			
FOXH1	70	22			
FOXI1	16	55			
FOXJ1	0	182		HIP	
FOXK2	0	0			
FOXL1	21	4			
FOXL2	23	14			
FOXM1	29	24			
FOXN2	187	202	SN	HIP	SN, HIP
FOXO3	0	0			
FOXO4	35	80			
FOXP2	89	94			
FOXP3	64	21			
FOXS1	14	6			
FUBP1	228	331	SN	HIP	SN, HIP
GABPA	0	0			
GABPB1	20	19			
GAS7	64	20			
GATA1	44	29			
GATA2	31	46			
GATA3	1	27			
GATA4	81	71			

GATA5	14	23			
GATA6	119	39	SN		
GATAD1	142	87	SN		
GBX1	94	60			
GBX2	8	10			
GCFC2	175	191	SN	HIP	SN, HIP
GCM1	65	14			
GLI2	43	21			
GLI3	10	72			
GLIS3	135	52	SN		
GMEB1	32	18			
GPBP1	41	111		HIP	
GSC	50	17			
GSC2	22	21			
GSX1	27	4			
GSX2	42	28			
GTF2H3	275	216	SN	HIP	SN, HIP
GTF2H4	8	1			
GTF2I	59	37			
GTF2IRD1	5	19			
HCFC1	115	95	SN		
HDAC1	89	55			
HDAC2	102	30	SN		
HDX	32	98			
HELT	6	3			
HES1	34	3			
HES6	24	35			
HESX1	0	0			
HEY1	86	86			
HEY2	71	12			
HEYL	25	5			
HHEX	3	4			
HIC1	50	46			
HIF1A	116	14	SN		
HIF3A	16	8			
HINFP	48	115		HIP	
HIRA	70	118		HIP	
HLF	75	190		HIP	
HLX	42	24			
HMBOX1	289	31	SN		
HMG20A	211	349	SN	HIP	SN, HIP
HMG20B	39	32			
HMGA1	44	46			
HMGB1	32	86			
HMGB2	0	8			
HMX1	15	19			
HMX3	1	8			
HNF1A	81	77			
HNF1B	96	9			
HNF4A	121	57	SN		
HNF4G	5	1			
HNRNPAB	42	145		HIP	
HOPX	1	45			
HOXA1	65	17			
HOXA10	32	38			
HOXA11	40	7			
HOXA13	10	6			
HOXA2	2	2			
HOXA3	22	33			
HOXA4	10	3			
HOXA5	17	16			
HOXA6	2	11			
HOXA7	3	16			
HOXA9	14	14			
HOXB1	8	15			
HOXB13	3	17			
HOXB2	8	1			
HOXB3	1	0			

HOXB4	19	18			
HOXB5	5	16			
HOXB6	12	4			
HOXB7	17	5			
HOXB8	5	1			
HOXB9	57	14			
HOXC10	12	10			
HOXC12	46	24			
HOXC13	44	34			
HOXC6	8	33			
HOXC8	60	40			
HOXC9	1	20			
HOXD1	0	0			
HOXD10	12	7			
HOXD11	30	11			
HOXD12	11	24			
HOXD13	1	2			
HOXD3	7	17			
HOXD4	55	23			
HOXD8	54	30			
HOXD9	26	25			
HR	8	18			
HSF1	14	11			
HSF2	233	31	SN		
HSF4	28	0			
HSF5	4	0			
ID1	10	4			
ID3	9	5			
IKZF1	77	43			
IKZF3	5	11			
IKZF4	65	17			
IRF2	24	50			
IRF3	84	7			
IRF4	23	8			
IRF5	7	4			
IRF6	16	26			
IRF9	0	0			
IRX1	21	85			
IRX2	8	65			
IRX3	88	14			
IRX4	17	23			
IRX5	55	43			
IRX6	20	4			
ISL1	9	59			
ISL2	111	5	SN		
ISX	7	17			
JDP2	3	8			
JUN	15	15			
JUNB	8	7			
JUND	11	18			
KAT7	242	139	SN	HIP	SN, HIP
KDM1A	243	190	SN	HIP	SN, HIP
KDM3A	102	57	SN		
KDM5A	180	218	SN	HIP	SN, HIP
KDM5B	105	52	SN		
KLF1	19	16			
KLF10	7	3			
KLF11	3	0			
KLF12	54	307		HIP	
KLF16	45	277		HIP	
KLF17	3	9			
KLF2	3	86			
KLF3	34	112		HIP	
KLF4	0	6			
KLF5	127	31	SN		
KLF7	175	108	SN	HIP	SN, HIP
KLF9	11	5			
KMT2A	0	0			

KMT2B	125	83	SN		
L3MBTL1	53	33			
L3MBTL4	37	82			
LBX1	6	20			
LBX2	80	23			
LEF1	40	23			
LEUTX	22	0			
LHX1	0	20			
LHX2	60	28			
LHX3	63	44			
LHX4	4	1			
LHX5	15	18			
LHX6	47	62			
LHX8	9	18			
LHX9	0	0			
LMO4	63	127		HIP	
LMX1A	6	72			
LZTR1	268	138	SN	HIP	SN, HIP
LZTS1	92	25			
MAF	1	15			
MAFA	0	54			
MAFB	9	4			
MAFF	13	13			
MAFG	50	86			
MAFK	1	0			
MAX	12	19			
MBD1	19	26			
MECOM	25	14			
MECP2	34	4			
MEF2A	41	146		HIP	
MEF2B	51	34			
MEF2C	21	78			
MEF2D	182	96	SN		
MEIS2	37	67			
MEIS3P1	0	0			
MEOX1	0	14			
MEOX2	0	0			
MESP1	3	20			
MESP2	1	13			
MGA	187	110	SN	HIP	SN, HIP
MKL1	19	28			
MKL2	41	110		HIP	
MLLT10	145	222	SN	HIP	SN, HIP
MLX	1	16			
MLXIPL	121	22	SN		
MMP14	12	26			
MNT	56	50			
MNX1	12	20			
MSC	13	77			
MSL3	32	1			
MSRB2	206	40	SN		
MTA1	101	17	SN		
MTA2	52	48			
MTA3	144	221	SN	HIP	SN, HIP
MTF1	10	15			
MXD1	13	43			
MYB	1	48			
MYBL2	28	196		HIP	
MYC	1	6			
MYCL	0	2			
MYF6	12	13			
MYNN	168	166	SN	HIP	SN, HIP
MYOG	83	41			
MYPOP	147	43	SN		
MYRF	172	205	SN	HIP	SN, HIP
MYRFL	0	0			
MYT1	44	6			
MYT1L	0	0			

MZF1	19	2			
NANOGNB	34	5			
NANOGP1	0	0			
NCOR1	86	100		HIP	
NEUROD1	1	102		HIP	
NEUROD2	96	164		HIP	
NEUROG1	11	21			
NFAT5	0	0			
NFATC1	55	47			
NFATC2	27	12			
NFATC3	47	77			
NFE2	0	0			
NFE2L1	106	135	SN	HIP	SN, HIP
NFE2L2	156	67	SN		
NFE2L3	32	60			
NFIA	89	69			
NFIC	47	11			
NFIL3	4	2			
NFIX	22	17			
NFKB1	143	180	SN	HIP	SN, HIP
NFKB2	34	31			
NFX1	117	204	SN	HIP	SN, HIP
NFXL1	297	254	SN	HIP	SN, HIP
NFYA	72	53			
NFYB	9	27			
NFYC	0	0			
NKX1-2	52	84			
NKX2-1	62	25			
NKX2-2	70	17			
NKX2-3	54	19			
NKX2-4	54	90			
NKX2-5	0	10			
NKX2-6	23	4			
NKX2-8	28	14			
NKX3-1	0	0			
NKX3-2	68	86			
NKX6-1	0	12			
NKX6-2	167	229	SN	HIP	SN, HIP
NKX6-3	34	18			
NME2	29	38			
NOBOX	64	48			
NOTCH1	81	101		HIP	
NPAS1	49	42			
NPAS2	0	7			
NPAT	458	201	SN	HIP	SN, HIP
NR0B2	0	3			
NR1H4	3	0			
NR1I3	19	60			
NR2C1	4	34			
NR2C2	56	89			
NR2F1	6	69			
NR2F6	98	38			
NR3C1	65	118		HIP	
NR3C2	37	181		HIP	
NR5A2	47	26			
NRL	23	6			
ONECUT3	1	6			
OTP	0	5			
OTX1	20	57			
OTX2	14	1			
PA2G4	1	86			
PAX3	0	10			
PAX4	32	55			
PAX5	16	23			
PAX6	0	0			
PAX7	14	175		HIP	
PAX8	23	25			
PAXBP1	84	151		HIP	

PBX1	299	41	SN		
PBX2	60	9			
PBX3	78	65			
PBX4	5	0			
PCGF2	73	9			
PCGF6	13	3			
PDX1	43	36			
PFDN1	0	0			
PGBD1	30	79			
PHF1	107	20	SN		
PHF5A	3	6			
PHOX2A	44	32			
PHOX2B	21	20			
PHTF1	320	118	SN	HIP	SN, HIP
PITX1	9	8			
PITX2	17	45			
PITX3	80	145		HIP	
PKNOX1	14	52			
PKNOX2	121	25	SN		
PLAG1	22	14			
PLAGL2	0	0			
POU1F1	1	0			
POU2F1	44	79			
POU2F2	3	5			
POU2F3	29	54			
POU3F1	31	61			
POU3F2	72	18			
POU3F3	12	21			
POU3F4	1	0			
POU5F1	0	0			
POU5F1B	0	0			
POU5F2	0	0			
POU6F1	40	81			
POU6F2	2	88			
PPARA	94	183		HIP	
PPARD	105	104	SN	HIP	SN, HIP
PPARG	29	58			
PRDM1	0	1			
PRDM2	71	42			
PROP1	61	23			
PROX1	32	69			
PRRX1	80	42			
PRRX2	19	23			
PTTG1	17	37			
PURB	0	0			
RARA	12	21			
RARG	2	11			
RAX	138	246	SN	HIP	SN, HIP
RAX2	159	92	SN		
RB1	122	179	SN	HIP	SN, HIP
RBPJ	110	89	SN		
RBPJL	72	48			
RCAN1	44	29			
RCOR2	0	13			
REL	73	96			
RELA	80	49			
RELB	72	26			
RERE	28	40			
REST	49	114		HIP	
REXO4	0	0			
RFX3	60	168		HIP	
RFX5	50	16			
RFXANK	9	25			
RFXAP	18	6			
RHOXF1	9	0			
RHOXF2	0	0			
RNF4	0	0			
RORA	36	29			

RORB	31	53			
RREB1	31	143		HIP	
RUNX1	81	65			
RUNX1T1	0	0			
RUNX2	45	30			
RUNX3	20	53			
RXRA	41	55			
RXRB	109	71	SN		
SALL1	0	0			
SALL2	95	18			
SATB1	52	110		HIP	
SATB2	26	27			
SCAND1	20	59			
SCAND2P	0	0			
SCAND3	284	391	SN	HIP	SN, HIP
SCMH1	25	24			
SCML1	13	8			
SCML2	14	18			
SCRT1	29	3			
SEBOX	1	0			
SHOX2	17	46			
SIM1	27	132		HIP	
SIM2	1	3			
SIN3A	30	68			
SIX1	6	9			
SIX2	15	7			
SIX4	5	0			
SIX5	70	22			
SIX6	0	11			
SLC26A3	160	31	SN		
SLC2A4RG	3	24			
SLC30A9	305	595	SN	HIP	SN, HIP
SMAD1	66	18			
SMAD2	11	89			
SMAD3	27	42			
SMAD5	166	133	SN	HIP	SN, HIP
SMAD6	11	3			
SMAD7	14	13			
SMAD9	51	47			
SNAI3	7	28			
SNAPC2	123	30	SN		
SNAPC4	137	23	SN		
SNAPC5	26	17			
SOHLH1	35	15			
SOX1	23	16			
SOX13	11	6			
SOX15	22	17			
SOX21	34	75			
SOX4	8	32			
SOX5	47	91			
SOX6	16	0			
SOX7	16	5			
SOX8	41	12			
SOX9	117	84	SN		
SP1	55	36			
SP140	13	67			
SP4	52	4			
SPDEF	41	49			
SPEN	46	90			
SPI1	100	81	SN		
SPIB	3	12			
SPIC	0	0			
SREBF1	62	30			
SREBF2	175	154	SN	HIP	SN, HIP
SRF	22	60			
ST18	217	139	SN	HIP	SN, HIP
STAT1	132	140	SN	HIP	SN, HIP
STAT2	218	123	SN	HIP	SN, HIP

STAT3	101	77	SN		
STAT4	114	66	SN		
STAT5A	4	44			
STAT5B	187	36	SN		
STAT6	16	25			
STRN3	337	346	SN	HIP	SN, HIP
SUPT4H1	296	244	SN	HIP	SN, HIP
SUPT6H	96	92			
T	2	19			
TADA2A	49	173		HIP	
TADA3	19	15			
TAF10	70	45			
TAF12	84	95			
TAF13	78	85			
TAF1B	259	61	SN		
TAF4	6	5			
TAF4B	397	70	SN		
TAF5	15	53			
TAF5L	6	23			
TAF6	134	151	SN	HIP	SN, HIP
TAF7	338	95	SN		
TAL1	12	19			
TARDBP	90	NA			
TBPL2	4	6			
TBR1	36	44			
TBX10	39	11			
TBX15	1	0			
TBX18	25	15			
TBX19	0	1			
TBX2	33	6			
TBX20	17	15			
TBX21	51	16			
TBX22	11	0			
TBX3	28	11			
TBX4	50	83			
TBX5	17	13			
TBX6	39	52			
TCEAL1	40	57			
TCF12	152	89	SN		
TCF15	11	36			
TCF19	8	25			
TCF20	74	90			
TCF25	400	82	SN		
TCF3	60	92			
TCF4	11	108		HIP	
TCF7	70	27			
TCF7L1	26	17			
TCF7L2	2	8			
TCFL5	106	59	SN		
TEAD2	17	3			
TEAD3	10	8			
TEAD4	7	4			
TEF	25	8			
TFAM	48	35			
TFAP2B	70	9			
TFAP2C	0	3			
TFAP2E	19	8			
TFCP2	44	37			
TFCP2L1	0	4			
TFDP1	17	12			
TFDP3	4	20			
TFE3	93	19			
TFEB	79	54			
TFEC	125	148	SN	HIP	SN, HIP
TGIF1	18	20			
TGIF2	10	0			
THRA	49	55			
THRB	63	128		HIP	

TLX1	46	75			
TLX2	176	167	SN	HIP	SN, HIP
TLX3	5	5			
TMEM229A	17	39			
TP53	0	0			
TP63	228	84	SN		
TP73	43	51			
TPRX1	0	0			
TRIM22	98	23			
TRIM25	0	0			
TRIM28	47	153		HIP	
TRIM29	13	18			
TRPS1	214	131	SN	HIP	SN, HIP
TSC22D1	325	443	SN	HIP	SN, HIP
TSC22D2	6	11			
TSC22D3	4	10			
TSC22D4	76	38			
TSHZ1	8	24			
TSHZ2	0	0			
TSHZ3	29	49			
TULP4	84	154		HIP	
TWIST2	0	0			
UBN1	7	12			
UBP1	46	64			
UHRF1	1	0			
UNCX	34	122		HIP	
USF2	0	0			
VAV1	99	66			
VAX1	2	18			
VAX2	41	30			
VDR	7	18			
VENTX	13	2			
VSX1	2	1			
VSX2	8	3			
WNT5A	50	27			
WT1	108	44	SN		
XBP1	33	13			
YBX3	2	19			
YEATS4	92	109		HIP	
YY1	35	222		HIP	
ZBTB17	8	24			
ZBTB18	17	108		HIP	
ZBTB25	56	29			
ZBTB38	37	67			
ZBTB48	2	4			
ZC3H8	22	23			
ZEB1	71	28			
ZEB2	79	92			
ZFHX3	54	59			
ZFHX4	119	57	SN		
ZFP36L1	43	79			
ZFP36L2	36	47			
ZFP37	24	17			
ZFP42	17	19			
ZFP69B	87	123		HIP	
ZGLP1	71	47			
ZGPAT	0	0			
ZHX1	71	31			
ZHX2	3	4			
ZHX3	28	45			
ZIC1	6	5			
ZIC2	12	15			
ZIC3	3	0			
ZIC5	0	0			
ZKSCAN1	69	65			
ZKSCAN3	2	2			
ZKSCAN4	0	0			
ZKSCAN5	23	51			

ZKSCAN8	80	59			
ZNF131	15	30			
ZNF132	38	10			
ZNF133	23	7			
ZNF134	66	53			
ZNF135	3	44			
ZNF138	0	0			
ZNF140	12	12			
ZNF148	232	208	SN	HIP	SN, HIP
ZNF154	103	31	SN		
ZNF155	0	0			
ZNF157	0	0			
ZNF165	0	0			
ZNF169	0	0			
ZNF174	53	89			
ZNF175	34	56			
ZNF189	92	87			
ZNF19	0	0			
ZNF197	44	58			
ZNF202	2	3			
ZNF207	164	110	SN	HIP	SN, HIP
ZNF213	4	12			
ZNF215	5	130		HIP	
ZNF217	127	32	SN		
ZNF219	136	33	SN		
ZNF232	23	2			
ZNF236	18	52			
ZNF24	30	41			
ZNF256	21	39			
ZNF263	1	23			
ZNF268	4	0			
ZNF274	13	2			
ZNF277	220	187	SN	HIP	SN, HIP
ZNF281	72	212		HIP	
ZNF287	36	78			
ZNF3	59	90			
ZNF33A	27	33			
ZNF35	0	0			
ZNF367	0	0			
ZNF37A	40	63			
ZNF394	6	55			
ZNF397	19	26			
ZNF41	77	86			
ZNF445	111	54	SN		
ZNF449	36	26			
ZNF45	41	2			
ZNF483	283	256	SN	HIP	SN, HIP
ZNF500	0	0			
ZNF518A	136	29	SN		
ZNF639	46	23			
ZNF69	0	0			
ZNF70	45	7			
ZNF71	0	3			
ZNF75D	5	20			
ZNF80	6	5			
ZNF81	18	18			
ZNF83	18	47			
ZNF85	0	0			
ZNF90	0	0			
ZNF92	2	28			
ZNF93	168	1	SN		
ZRANB2	281	254	SN	HIP	SN, HIP
ZSCAN10	9	10			
ZSCAN12	23	22			
ZSCAN16	3	6			
ZSCAN2	0	13			
ZSCAN20	19	0			
ZSCAN21	29	49			

ZSCAN22	3	1			
ZSCAN23	46	7			
ZSCAN25	1	0			
ZSCAN26	42	49			
ZSCAN30	10	56			
ZSCAN31	0	1			
ZSCAN4	35	12			
ZSCAN9	25	16			
ZXDC	0	0			
GATAD2A	NA	151		HIP	
SMAD4	NA	51			
ZXDA	NA	0			

Supplementary Table 2: MRs in PD identified with MRA

Transcription Factor	Regulon size	Adjusted *p-value*
SCAND3	386	2.4e-52
SLC30A9	595	1.5e-49
TSC22D1	438	4.9e-36
CERS6	242	1.3e-33
CSRNP2	410	1.6e-30
FBXW7	281	4.7e-27
CNOT7	254	3.1e-22
ATF2	248	5.8e-21
SUPT4H1	244	4.0e-15
KLF12	305	8.0e-14
HMG20A	348	9.5e-13
ECSIT	210	6.8e-11
ZNF483	255	9.6e-11
RREB1	142	1.5e-10
FOXC2	180	5.7e-10
PHTF1	118	6.7e-10
BCL3	213	8.2e-10
ZNF215	130	3.2e-09
ST18	138	3.7e-09
ZFP69B	123	1.1e-08
STAT1	140	1.9e-08
CBFB	132	4.9e-08
MEF2A	146	9.4e-08
NKX6-2	227	4.9e-07
CEBPG	126	1.7e-06
FOXN2	202	2.6e-06
YEATS4	109	3.1e-06
HLF	187	3.6e-06
STAT2	123	5.1e-06
KDM1A	190	5.9e-06
TCF4	108	1.6e-05
STRN3	346	1.9e-05
BTAF1	237	2.0e-05
MGA	110	2.4e-05
TAF6	150	4.3e-05
MTA3	220	8.6e-05
ADNP2	154	8.8e-05
PAXBP1	150	9.0e-05
PPARD	104	9.1e-05
CITED2	123	0.00015
ZBTB18	107	0.00017
CERS2	113	0.00024
YY1	222	0.00035
LMO4	127	0.00065
THRB	128	0.00075
RFX3	165	9.0e-04
KAT7	139	0.001
GTF2H3	215	0.0014
NFXL1	254	0.0015
ELF1	144	0.0021

Supplementary Table 3: MRs in PD identified with GSEA

Transcription Factor	Regulon size	Adjusted *p-value*
SCAND3	386	0.003224
CERS6	242	0.003224
TSC22D1	438	0.003224
ZFP69B	123	0.003224
RREB1	142	0.003224
HLF	187	0.003224
ZNF215	130	0.003224
FBXW7	281	0.003224
CITED2	123	0.003224
ZBTB18	107	0.003224
PHTF1	118	0.003224
NKX6-2	227	0.003224
CNOT7	254	0.003224
ATF2	248	0.003224
CSRNP2	410	0.003224
ST18	138	0.003224
KLF12	305	0.003224
SLC30A9	595	0.003224
CBFB	132	0.003224
SATB1	110	0.011349
ECSIT	210	0.003224
ZNF483	255	0.003224
SUPT4H1	244	0.003224
LMO4	127	0.039405
MTA3	220	0.011349
FOXC2	180	0.0092516
HMG20A	348	0.024552

CTBP1	104	0.0021
SREBF2	154	0.0023
HIRA	118	0.0023
ZRANB2	254	0.0039
NR3C1	118	0.0045
ZNF281	212	0.0045
REST	114	0.0047
SATB1	110	0.0051
NFKB1	180	0.0051
BRD8	191	0.0059
ZNF277	187	0.011
GPBP1	111	0.011
KLF3	112	0.012
NR3C2	180	0.013
NCOR1	100	0.015
PPARA	182	0.016
HNRNPAB	145	0.019
NFX1	204	0.019
GCFC2	191	0.035
NPAT	201	0.035
LZTR1	138	0.041

Table 4: MRs in AD identified with MRA

Transcription Factor	Regulon size	Adjusted p-value
SLC30A9	595	7.6e-78
SCAND3	386	9.5e-51
CSRNP2	410	1.1e-47
TSC22D1	438	1.3e-41
ATF2	248	1.7e-40
CNOT7	254	3.8e-35
KLF12	305	5.8e-31
HMG20A	348	1.4e-30
ZNF483	255	5.6e-29
STRN3	346	1.1e-27
HNRNPAB	145	2.1e-27
ZNF281	212	7.6e-27
STAT1	140	1.1e-26
YY1	222	7.8e-26
NFXL1	254	1.8e-24
FOXC2	180	4.2e-24
CERS6	242	5.2e-24
CEBPG	126	1.7e-22
ZNF148	208	3.8e-22
SUPT4H1	244	2.3e-21
ZNF277	187	3.3e-21
FUBP1	331	1.0e-20
ADNP2	154	6.7e-20
KAT7	139	6.4e-19
NPAT	201	2.4e-18
FBXW7	281	2.9e-18
PHTF1	118	1.8e-17
GTF2H3	215	6.3e-17

Table 5: MRs in AD identified with GSEA

Transcription Factor	Regulon size	Adjusted p-value
RREB1	142	0.0088911
ZBTB18	107	0.011855
SCAND3	386	0.0088911
CERS6	242	0.0088911
HLF	187	0.011855
TSC22D1	438	0.0088911
CNOT7	254	0.0088911
ZNF215	130	0.02092
MTA3	220	0.0088911
ZFP69B	123	0.011855
CSRNP2	410	0.0088911
KLF12	305	0.0088911
FBXW7	281	0.0088911
ZNF483	255	0.016671
YY1	222	0.011855
ATF2	248	0.011855
NKX6-2	227	0.039516
SLC30A9	595	0.0088911

KDM1A	190	1.5e-16
AFF4	136	2.1e-16
TULP4	154	1.4e-15
MEF2A	146	1.8e-15
NFX1	204	1.1e-14
ATF6	154	1.8e-14
ECSIT	210	2.9e-14
TRIM28	153	4.1e-14
ZNF215	130	4.6e-14
BTAF1	237	4.6e-14
CBL	169	6.0e-14
GPBP1	111	2.7e-13
BLZF1	172	6.3e-13
BRD8	191	6.2e-12
ZRANB2	254	7.5e-12
HIRA	118	7.5e-12
MTA3	220	4.1e-11
LZTR1	138	2.0e-10
ZFP69B	123	2.2e-10
NFE2L1	135	6.4e-10
TCF4	108	2.7e-09
BCL3	213	3.8e-09
GCFC2	191	6.0e-09
RB1	178	6.6e-09
YEATS4	109	1.2e-08
ARID4A	164	9.3e-08
SREBF2	154	9.4e-08
RREB1	142	1.4e-07
MYNN	166	8.9e-07
SMAD5	132	9.1e-07
PAXBP1	150	1.2e-06
MGA	110	1.3e-06
NFKB1	180	4.2e-06
FOXN2	202	4.5e-06
NR3C1	118	4.8e-06
TAF6	150	6.1e-06
CREB3	109	6.3e-06
NCOR1	100	8.3e-06
ZBTB18	107	9.0e-06
PPARD	104	9.8e-06
C1orf85	108	1.2e-05
ZNF207	108	2.9e-05
KDM5A	218	7.4e-05
MKL2	109	8.7e-05
TADA2A	172	0.00012
KLF16	276	0.00013
HLF	187	0.00022
KLF7	108	0.00034
CTBP1	104	0.0029
LMO4	127	0.003
GATAD2A	151	0.0034
RFX3	165	0.0046
TRPS1	130	0.0047
STAT2	123	0.0052
CBFB	132	0.0062
DRAP1	106	0.013

HINFP	115	0.016
THRB	128	0.018
NKX6-2	227	0.019
NR3C2	180	0.025
MLLT10	221	0.048

V. Conclusões e perspectivas

A grande complexidade do cérebro deve-se à presença de tipos celulares e mecanismos moleculares específicos nas suas diversas regiões, que interagem para possibilitar a emergência de processos cognitivos superiores. Essa heterogeinicidade, por sua vez, é possível apenas pela ação diferencial de fatores de transcrição, que foram alvo de nossas análises. No presente trabalho, redes de regulação transcricionais do hipocampo e *substância nigra* foram moduladas e fatores de transcrição importantes para a caracterização de cada uma das estruturas foram identificados. Esse dado é de grande relevância para a compreensão das bases moleculares que controlam o desenvolvimento e as funções do sistema nervoso central.

Ainda, a partir da rede inferida para cada estrutura e dados de microarranjo de indivíduos saudáveis e acometidos por pelas doenças de Parkinson e Alzheimer, propusemos TFs que agem como reguladores mestres nessas desordens. A identificação das mudanças de expressão gênica que ocorrem em situações patológicas possibilita a compreensão de processos que subjazem a degeneração e, assim, direciona a busca por novos fármacos capazes de reverter esse padrão.

Com isso em mente, o trabalho tem como principais perspectivas:

- Utilizar mais dados de estudos caso-controle adquiridos do GEO para a determinação de reguladores mestres, conferindo mais confiabilidade e robustez aos resultados aqui obtidos;
- Validar alvos selecionados do presente trabalho em biópsias de tecido cerebral *post-mortem* derivado de pacientes com DA e DP, através da análise imunohistoquímica semi-quantitativa, *western blot* e proteômica;
- Validar alvos selecionados em modelos celulares da DA e DP, através de experimentos de imunoprecipitação da cromatina (ChIPseq), perturbações por silenciamento e proteômica;

- Buscar, através de análise de mapas de conectividade, selecionar o perfil de fármacos que antagonisam as assinaturas moleculares das DP e DA;
- Determinar o real efeito e a eficácia das drogas obtidas a partir das análises de bioinformática em modelos celulares das doenças.

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VII. Anexo – Formatação da revista *Brain Research*

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