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Gene co-expression networks shed light into diseases of brain iron accumulation

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ABSTRACT

Aberrant brain iron deposition is observed in both common and rare neurodegenerative disorders, including those categorized as Neurodegeneration with Brain Iron Accumulation (NBIA), which are characterized by focal iron accumulation in the basal ganglia. Two NBIA genes are directly involved in iron metabolism, but whether other NBIA-related genes also regulate iron homeostasis in the human brain, and whether aberrant iron deposition contributes to neurodegenerative processes remains largely unknown. This study aims to expand our understanding of these iron overload diseases and identify relationships between known NBIA genes and their main interacting partners by using a systems biology approach.

We used whole-transcriptome gene expression data from human brain samples originating from 101 neuropathologically normal individuals (10 brain regions) to generate weighted gene co-expression networks and cluster the 10 known NBIA genes in an unsupervised manner. We investigated NBIA-enriched networks for relevant cell types and pathways, and whether they are disrupted by iron loading in NBIA diseased tissue and in an in vivo mouse model.

We identified two basal ganglia gene co-expression modules significantly enriched for NBIA genes, which resemble neuronal and oligodendrocytic signatures. These NBIA gene networks are enriched for iron-related genes, and implicate synapse and lipid metabolism related pathways. Our data also indicates that these networks are disrupted by excessive brain iron loading.

We identified multiple cell types in the origin of NBIA disorders. We also found unforeseen links between NBIA networks and iron-related processes, and demonstrate convergent pathways connecting NBIAs and phenotypically overlapping diseases. Our results are of further relevance for these diseases by providing candidates for new causative genes and possible points for therapeutic intervention.

1. Introduction

Aberrant brain iron deposition occurs in common neurodegenerative disorders (e.g. Parkinson's and Alzheimer's diseases (Oakley et al., 2007; Tao et al., 2014)), and more prominently in rare inherited diseases categorized as Neurodegeneration with Brain Iron Accumulation (NBIA) (Dusek et al., 2012). Iron is essential for normal brain function and is heterogeneously and dynamically distributed in the brain (Piñero and Connor, 2000; Rouault, 2013). The basal ganglia are among the regions with highest iron levels, and the highest concentrations are observed in oligodendrocytes. Our understanding of brain iron metabolism and how it relates to neurodegeneration and disease is limited due to the inability to distinguish brain cell types via non-invasive techniques (e.g. MRI) and poor understanding of how iron traffics in

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the brain to adequately supply neurons, astrocytes, oligodendrocytes and microglia (Rouault, 2013; Kruer et al., 2012; Schneider et al., 2013; Levi and Finazzi, 2014).

NBIA disorders are clinically characterized by a progressive movement disorder with complicating symptoms that can vary significantly in terms of range and severity, and frequently include neuropsychiatric disturbances, such as cognitive deficits, personality changes with impulsivity and violent outbursts, depression, emotional lability, and obsessive compulsive disorder (Gregory et al., 2009). This clinically heterogeneous picture is unified by focal brain iron accumulation, predominantly in the basal ganglia (Kruer et al., 2012; Schneider et al., 2013; Levi and Finazzi, 2014). Ten NBIA genetic diseases have already been defined (Table 1), yet many cases remain genetically undiagnosed (Levi and Finazzi, 2014). Two NBIA genes (*FTL* and *CP*) are directly involved in iron metabolism, but it remains elusive whether other NBIA genes also regulate iron-related processes in the human brain.

We analyzed whole-transcriptome gene expression data from normal human brain and used weighted gene co-expression network analysis (WGCNA) to group NBIA genes into modules in an unsupervised manner (Langfelder and Horvath, 2008; Oldham et al., 2006, 2008; Zhang and Horvath, 2005). This systems-biology approach (Lee et al., 2004; Stuart et al., 2003) enables the identification of modules of biologically related genes that are co-expressed and co-regulated (Oldham et al., 2008; Konopka, 2011; Rosen et al., 2011; Winden et al., 2009), and can give insights on cell-specific molecular signatures (Oldham et al., 2008; Bettencourt et al., 2014; Forabosco et al., 2013). The main goal of this study was to expand our understanding of these iron overload diseases by identifying relationships and shared molecular pathways between known NBIA genes, and unraveling transcriptionally linked novel candidates to facilitate discovery of new genes associated with these diseases and of possible entry points to therapeutic intervention.

2. Subjects and methods

2.1. Human control brain samples and whole-genome expression profiling

Brain samples from 101 adult individuals were collected by the Medical Research Council (MRC) Sudden Death Brain and Tissue Bank (Millar et al., 2007). All brains samples were neuropathologically normal, had fully informed consent and were authorized for ethically approved scientific investigation (Research Ethics Committee 10/H0716/3). Within the frame of the UK Brain Expression Consortium (UKBEC), total RNA was isolated and processed for analysis using Affymetrix Exon 1.0 ST Arrays (Affymetrix UK Ltd., High Wycombe, UK) as described elsewhere (Bettencourt et al., 2014; Forabosco et al., 2013; Trabzuni et al., 2011).

2.2. Weighted gene co-expression network analysis in the adult normal human brain

Using whole-transcriptome gene expression data, NBIA genes/transcripts were assigned to co-expression modules (arbitrary colors) identified through WGCNA (Langfelder and Horvath, 2008; Zhang and Horvath, 2005). For the adult brain network analysis (10 brain regions from 101 adult individuals, UKBEC data) a total of 15,409 transcripts (13,706 genes) passing quality control were used to identify modules, and 3743 additional transcripts (3541 genes) were assigned to modules based on their highest module membership, as previously described (Forabosco et al., 2013). Briefly, the WGCNA network was constructed for each tissue using a signed network with power (Beta) of 12 to achieve a scale-free topology. A dissimilarity matrix based on topological overlap measure (TOM) was used to identify gene modules (i.e. densely interconnected and co-expressed genes), through a dynamic tree-cutting algorithm. More details are given by Forabosco et al. (2013).

Module preservation statistics (Z summary) were calculated as previously described (Langfelder et al., 2011) to assess how well modules from one tissue are preserved in another tissue. Based on the empirical thresholds proposed by Langfelder et al. (Langfelder et al., 2011), Z summary scores above 10 indicate strong evidence for module preservation across brain regions. To determine the relevance of each gene in a module, we estimated the module membership (MM), also known as eigengene-based connectivity. Gene interconnections within NBIA transcript-enriched modules were further investigated using VisANT (http://visant.bu.edu) (Hu et al., 2004).

Hypergeometric distribution was used to evaluate the overrepresentation of NBIA and iron-related gene transcripts in the gene co-expression modules (nominal p-values <0.05 were considered significant). To further assess the statistical significance of the enrichment of NBIA genes in given putamen modules, we developed a permutation test to estimate the probability that g genes will be found together by chance within a module of size equal or less than m for a given partition of genes $G = \{g_1,\ldots, g_n\}$, arranged into k modules $P = \{p_1,\ldots,p_k\}$, such that each gene g_i belongs only to a single module. To estimate the probability of finding g genes in a module of size m or less in partition P, we randomly permuted the genes in G in a list and annotated each gene in that list with the module in P to which the gene belongs. Then we repeated the following procedure 10^6 times, randomly choosing g positions from the list and checking whether the corresponding genes were annotated with the same module and the module had size m or less. Finally, the probability of finding by chance g genes in a module of size m or less was estimated by dividing by 10^6 the number of times g genes were found together in such modules.

2.3. Validation of basal ganglia co-expression networks in independent data sets

We used independent and publicly available basal ganglia gene expression networks (Oldham et al., 2008), from 27 adult caudate nucleus samples, to investigate whether our NBIA-containing modules overlap with modules in those previously published networks. We also used the only publicly available basal ganglia pediatric whole-transcriptome gene expression data set (Kang et al., 2011) (7 striatum samples from clinically unremarkable donors with ages ranging from 2 to 19 years) to perform WGCNA (Langfelder and Horvath, 2008; Zhang and Horvath, 2005). We generated pediatric signed networks using a power (Beta) of 33 and a height of 0.2. A total of 15,285 genes passing quality control were used to identify modules. Fisher's exact test was used to determine the significance of the overlap between distinct networks (nominal $p < 0.05$ was considered significant).

2.4. Gene expression analysis in NBIA diseased basal ganglia tissue

Additional validation studies investigated whether the NBIA-containing modules overlap with differentially expressed genes in human NBIA disorders. We used post-mortem basal ganglia tissue from two adults, one male and one female (66 and 81 years at death, respectively), with a confirmed clinicopathological diagnosis of NBIA (Canadian Brain Tissue Bank, University of Toronto, Canada), and two age- and gender-matched adults with no diagnosed neurological conditions (Newcastle Brain Tissue Resource, University of Newcastle, UK). All brain tissue was obtained with fully informed consent and the study was approved by the Human Research Ethics Committee of the University of Newcastle, Australia (H-2010-1219). Total RNA was obtained as previously described (Johnstone et al., 2012; Acikyol et al., 2013), and arrays performed using the Illumina HumanHT-12 v4 Expression BeadChip (Illumina, San Diego, USA). Following Cubic Spline normalization in GenomeStudio Gene Expression Module (Illumina, v2010.3), genes were considered differentially expressed if the fold-change of the mean NBIA signal relative to the mean control signal for