1 Cholinergic circuit genes in the healthy brain are

2 differentially expressed in regions that exhibit gray

3 matter loss in Parkinson's disease

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20 **Running title:** Transcriptomics of gray matter loss in Parkinson's disease

21 **Abstract**

22 Structural covariance networks are able to identify functionally organized brain regions by gray matter

23 volume covariance. In Parkinson's disease, the posterior cingulate network and anterior cingulate

24 network showed decreased gray matter and therefore we examined the underlying molecular processes

25 of these anatomical networks in the healthy brain. Whole brain transcriptomics from post-mortem

26 samples from healthy adults, revealed upregulation of genes associated with serotonin, GPCR, GABA,

27 glutamate, and RAS signaling pathways in these PD-related regions. Our results also suggest involvement

28 of the cholinergic circuit, in which genes *NPPA*, *SOSTDC1*, and *TYRP1* may play a protective role.

29 Furthermore, both networks were associated with memory and neuropsychiatric disorders that overlap

30 with Parkinson's disease symptoms. The identified genes and pathways contribute to healthy functions

31 of the posterior and anterior cingulate networks and disruptions to these functions may in turn

32 contribute to the pathological and clinical events observed in Parkinson's disease.

33

Introduction

36

37 Parkinson's disease (PD) is a neurodegenerative disorder pathologically defined by the loss of

38 dopaminergic neurons in the substantia nigra and the presence of Lewy bodies in selective brain regions

39 (Goedert et al. 2012). Clinical symptoms involve impairment of diverse motor and non-motor symptoms

40 that get progressively worse over time. The decline in clinical performance has been associated with

41 changes in morphological properties of structural and functional neuroimaging networks (Lucas-Jiménez

42 et al. 2016; Wang et al. 2016; de Schipper et al. 2017). In turn, studies have investigated the relationship

43 between these imaging networks and genetic variants in PD-related genes to provide new insights into

44 the pathogenesis of PD (van der Vegt et al. 2009). However, less is known about the functional pathways

45 that underlie the spatial organization of brain regions contributing to PD. To identify the molecular

46 mechanisms underlying changes in structural and functional networks in PD, imaging data has been

47 integrated with brain wide expression atlases of the healthy brain (Hawrylycz et al. 2015; Arnatkevičiūtė

48 et al. 2019). Regional brain atrophy in PD patients was found to be correlated with the expression of

49 genes implicated in trans-synaptic alpha-synuclein transfer (Freeze et al. 2018) and a loss of regional

50 connectivity in PD patients was correlated with the regional expression of *MAPT* in the healthy brain

51 (Rittman et al. 2016). These studies show that combining imaging data in PD and gene expression from

52 the healthy brain can shed light on the molecular mechanisms underlying the observed differences

53 between PD and controls.

54 Structural covariance networks (SCNs) can reveal functional network organizations by identifying brain

55 regions that co-vary in gray matter volume across a population (Alexander-Bloch, Giedd, et al. 2013).

56 SCNs have been shown to be dysregulated in different neurological disorders (Alexander-Bloch,

57 Raznahan, et al. 2013; Spreng and Turner 2013; Coppen et al. 2016; Huang et al. 2017; Liu et al. 2019),

58 and variations in SCNs can be explained by transcriptomic similarity and structural connectivity (Romero-

59 Garcia et al. 2018; Yee et al. 2018). Hafkemeijer *et al.* (Hafkemeijer et al. 2014) identified nine SCNs

60   based on gray matter variation among healthy middle-aged to older adults. Gray matter volume in four

61   of these nine networks was negatively associated with age: a subcortical network, sensorimotor

62   network, posterior cingulate networks, and anterior cingulate network. Two of these networks were

63   found to show loss of gray matter volume in PD patients beyond the effects of aging: the posterior

64   cingulate network and anterior cingulate network (de Schipper et al. 2017). These two networks were

65   negatively associated with cognitive impairment and daytime sleepiness, respectively. Yet, it is unclear

66   which molecular mechanisms contribute to these differences in SCNs in individuals with PD.

67   In this study, we aim to identify the molecular mechanisms within the healthy brain underlying the loss

68   of integrity and atrophy in the anterior and posterior cingulate networks in PD patients. By integrating

69   the nine SCNs with spatial gene expression data from the Allen Human Brain Atlas, we investigate

70   whether expression patterns in the normal healthy brain are associated with patterns of gray matter

71   loss in PD. We show that genes highly expressed in the posterior and anterior cingulate networks were

72   associated with multiple neurotransmitter signaling pathways and involved in memory-related, pain-

73   related, and neuropsychiatric disorders. In addition, both anatomical networks showed high expression

74   of cholinergic gene markers known to act as regulators of extracellular signaling. Our results provide

75   new insights into the molecular processes underlying anatomical network function and aids in

76   understanding the selective progression of PD.

Materials and Methods

77

Transcriptomic data preprocessing

78

79   To understand transcriptomic signatures of nine anatomical networks of the healthy brain, we used

80   normalized gene expression data from the Allen Human Brain Atlas (AHBA), a post-mortem microarray

81   data set of 3,702 anatomical brain regions from six healthy individuals (5 males and 1 female, mean age

82   42, range 24–57 years) (Hawrylycz et al. 2015). We downloaded the data from http://human.brain-

83   map.org/. To filter and map probes to genes, the data was concatenated across the six donors. We

84   removed 10,521 probes with missing Entrez IDs, and 6,068 probes with low presence as they were

85   expressed above background in <1% of samples (PA-call containing presence/absence flag) (Hawrylycz et

86   al. 2015). The remaining 44,072 probes were mapped to 20,017 genes with unique Entrez IDs using the

87   *collapseRows*-function in R-package WGCNA v1.64.1 (Langfelder and Horvath 2008) as follows: i) if there

88   is one probe, that one probe is chosen, ii) if there are two probes, the one with maximum variance

89   across all samples is chosen (method="maxRowVariance"), iii) if there are more than two probes, the

90   probe with the highest connectivity (summed adjacency) is chosen (connectivityBasedCollapsing=TRUE).

91   For visualization of gene expression in heatmaps, data was Z-score normalized across all samples for

92   each brain donor separately. Heatmaps were plotted using R-package ComplexHeatmap v2.0.0 (Gu et al.

93   2016). Genes were clustered using complete linkage with Euclidean distances. The same color scale was

94   used for all heatmaps.

Mapping AHBA samples to SCNs of the healthy brain

96   We focused on anatomical networks that were previously defined based on whole brain gray matter

97   volume covariation in 370 middle-aged to older adults between 45 and 85 years; for more detailed

98   information on the networks see Hafkemeijer et al. 2014. Nine networks were defined and named

99   according to the presence of the main structures: thalamus (network A), lateral occipital cortex (network

100   B), posterior cingulate cortex (network C), anterior cingulate cortex (network D), temporal pole (network

101   E), putamen (network F), and cerebellum (networks G, H, and I). The same networks were previously

102   investigated for loss of integrity in 159 PD patients from the same age range, where the posterior

103   cingulate network (C) and anterior cingulate network (D) showed decreased gray matter; for

104 demographic and clinical information see de Schipper et al. 2017. All samples from each one of the six

105 donors in AHBA were mapped to regions defined by the nine SCNs in MNI coordinate space. The

106 mapping divided all the samples into two sets depending on their position inside (1) or outside (0) the

107 SCN mask.

108 **Differential expression analysis**

109 Differential expression analysis was performed between each of the two PD-related networks (posterior

110 and anterior cingulate cortex) and the other 7 non-PD-related networks together. A two-tailed t-test

111 was used for each gene and the analysis was done separately for each donor from AHBA. Since the

112 microarray data was log_2-transformed, the mean expression difference is interpreted as the log_2-

113 transformed fold-change (FC). The effect sizes for each one of the six donors were combined by meta-

114 analysis (metafor R-package 2.0). For the meta-analysis, a random effects model was applied which

115 assumes that each brain is considered to be from a larger population of brains and therefore takes the

116 within-brain and between-brain variance into account. The between-brain variance (tau^2) was estimated

117 with the Dersimonian-Delaird model. Variances and confidence intervals were obtained using the *escalc*-

118 function. The significance of summary effect sizes was assessed through a two-sided t-test (H_0: FC=0;

119 unequal variances). *P*-values of the effect sizes were Benjamini-Hochberg (BH) corrected for all 20,017

120 genes.

121 **Pathway analysis**

122 Pathway analysis was done with the ReactomePA R-package version 1.28 using the function

123 *enrichPathway* searching for human pathways. All 20,017 genes in the AHBA dataset were set as

124 background genes. Pathways with a minimum size of 10 genes were significant when BH-corrected $P <$

125 0.05.

126 **Cell-type marker enrichment**

127 Gene markers for 28 cell-types were downloaded from the Neuroexpresso database

128 (http://neuroexpresso.org/) using markers from all brain regions. These have been identified in a cross-

129 laboratory dataset of cell-type specific transcriptomes from the mouse brain (Mancarci et al. 2017). To

130 assess their expression, Entrez IDs of the mouse cell-type specific markers were converted to human

131 homologs (homologene R-package version 1.4) and filtered for genes present in the AHBA dataset

132 (Supplementary Table 1). Two markers with different mouse gene IDs (14972, *H2-K1*, microglial, and

133 15006, *H2-Q1* serotonergic), were converted to the same human gene ID (3105, *HLA-A*), and therefore

134 removed before analysis. For cell-type enrichment, we assessed which cell-type markers were

135 overrepresented among the differentially expressed genes. For 17 cell-types that had at least six

136 markers (astrocyte, Bergmann, cerebellar granule, dentate granule, ependymal, GabaReln,

137 hypocretinergic, microglia, activated microglia, deactivated microglia, noradrenergic, oligo, purkinje,

138 serotonergic, spinal cord cholinergic, spiny, and thalamus cholinergic), we assessed the significance with

139 the hypergeometric test and *P*-values were corrected for all 17 cell-types (BH-corrected $P < 0.05$).

140 **Enrichment of disease-associated genes**

141 Differentially expressed genes were also assessed for overrepresentation of disease-associated genes

142 from DisGeNET (Piñero et al. 2017). A table of 628,685 gene-disease associations were obtained from

143 DisGeNET version 6.0 (July, 2019) from http://www.disgenet.org/. A hypergeometric test was used to

144 assess the significance of overlapping genes ($P < 0.05$), and *P*-values were BH-corrected for 24,166

145 diseases. The odds ratio (OR) for cell-type and disease enrichment was calculated using the DescTools R-

146 package.

Results

Transcriptomics of the posterior and anterior cingulate networks

We analyzed the transcriptomes of healthy subjects across nine anatomical networks defined by structural covariance of gray matter volume among healthy middle-aged to older adults (Hafkemeijer et al. 2014). We focused on the posterior cingulate network and the anterior cingulate network that showed loss of gray matter in PD patients which seemed to be associated with cognition and excessive daytime sleepiness (Figure 1) (de Schipper et al. 2017). For this we used the AHBA microarray dataset of spatial gene expression in post-mortem brains. AHBA samples were mapped to each one of the nine networks A-I (Table 1). We characterized the transcriptional signature of the two PD-related networks (Network C and Network D) in these AHBA samples by comparing their gene expression pattern to the remaining seven networks together (non-PD-related).

Genes were differentially expressed within the posterior cingulate network or the anterior cingulate network compared to the other networks combined when absolute fold-change (FC) > 1 and Benjamini-Hochberg (BH) corrected P-value < 0.05. Differential expression analysis showed a large overlap of genes that were differentially expressed in the same direction in the two networks. We found that 73 genes in the posterior cingulate network and 39 genes in anterior cingulate network were downregulated, of which 25 genes overlapped between both networks (Figure 2A and B, Supplementary Table 2 and 3). Furthermore, 200 genes in the posterior cingulate network and 269 genes in anterior cingulate network were upregulated, for which 144 genes overlapped (Supplementary Table 4 and 5). Among the differentially expressed genes in the posterior and anterior cingulate networks, we further assessed the presence of PD-implicated genes identified in familial and genome-wide association studies (Bonifati 2014; Nalls et al. 2014; Chang et al. 2017). Although mutations in these genes may impact disease

169   outcome, none of these genes were differentially expressed in the posterior cingulate network and

170   anterior cingulate network.

171   For functional interpretation, differentially upregulated genes were further assessed for enrichment of

172   genes involved in pathways (Supplementary Table 6). As both networks shared many differentially

173   expressed genes, they also shared similar pathways involved in transcriptional regulation by *MECP2*,

174   GPCR signaling, voltage gated potassium channels, and neurotransmitter receptor and postsynaptic

175   signal transmission (Figure 2C). The associated pathways were hierarchically related to each other based

176   on the ontology of the Reactome Pathway Database. The posterior cingulate network was additionally

177   related to more specific pathways such as lysosphingolipid and LPA receptors, GABA receptor activation,

178   RAS-signaling mediated by NMDA receptors, glutamate binding, activation of AMPA receptors and

179   synaptic plasticity, and long-term potentiation. The anterior cingulate cortex was additionally associated

180   with serotonin receptors.

181   **Cell-type enrichment in anatomical networks**

182   The composition of specific cell-types can shape the transcriptomic features of anatomical networks.

183   Therefore, we analyzed whether genes differentially expressed in the posterior and anterior cingulate

184   networks were enriched for cell-type specific marker genes. Based on the average expression of marker

185   genes of a cell-type, the posterior and anterior cingulate networks showed high expression of gene

186   markers for brainstem cholinergic cells, GabaSSTReln, GabaVIPReln, glutamatergic, and pyramidal cells

187   (Figure 3 and Supplementary Figure 1).

188   Among the differentially upregulated genes in the posterior and anterior cingulate networks, we found

189   10 marker genes representing six cell-types: astrocyte, Bergmann, GabaVIPReln, hypocretinergic,

190   pyramidal, and thalamus cholinergic (

191   Table 2). Those that were significantly upregulated in the posterior cingulate network were also

192   significantly upregulated in the anterior cingulate network. In both networks, the 10 markers were

193   highly expressed in cortical regions, including the cingulate gyrus, and lowly expressed in limbic regions

194   (Figure 4 and Supplementary Figure 2).

195   Only genes upregulated in the anterior cingulate gyrus were significantly enriched for a cell-type, namely

196   thalamus cholinergic cells (OR = 17.12 and P = 2.01e-02). The responsible markers *NPPA*, *SOSTDC1*, and

197   *TYRP1* showed high expression within the anterior cingulate gyrus network, as well as in most parts of

198   the posterior cingulate gyrus network (Figure 4). Additionally, we showed that their expression was low

199   in limbic samples, including the thalamus, and high in cortical samples within both networks.

200   Interestingly, other thalamus cholinergic marker genes showed opposite expression patterns with high

201   expression in limbic samples and low expression in cortical samples (Supplementary Figure 3).

PD-related networks are transcriptionally associated to other brain diseases

203   Dysregulation of functional networks may result in a broader spectrum of disorders than PD. In addition,

204   PD comprises a spectrum of disorders that may result from network dysfunction. Therefore, we

205   assessed which disease-associated genes from DisGeNET were overrepresented among the differentially

206   upregulated genes in the posterior cingulate network as well as the anterior cingulate network. Since

207   both networks shared many upregulated genes, similar disease-associations were also found. We find

208   that genes upregulated in both networks were significantly associated with epileptic and non-epileptic

209   seizures, many mental disorders (bipolar, panic, autistic, cocaine-related, (age-related) memory, mood,

210   major depressive, and anxiety disorder), pain and schizophrenia (Figure 5). The posterior cingulate

211   network was more related to memory and pain-related disorders, while the anterior cingulate network

212   was more related to mental and neuropsychiatric disorders.

213   # Discussion

214   The posterior and anterior cingulate networks have been associated earlier with decreased gray matter

215   in PD patients. We examined transcriptomic signatures of both networks in the healthy brain to identify

216   molecular mechanisms underlying gray matter loss in PD. Pathway analysis revealed genes related to

217   gPCR signaling, transcriptional regulation by *MECP2*, and neurotransmitter receptors and postsynaptic

218   signal transmission. We only found significant enrichment of cell-types for genes upregulated in the

219   anterior cingulate gyrus, which were the thalamus cholinergic marker genes. Upon further examination

220   the specific genes were also highly expressed in the posterior cingulate cortex, although not

221   significantly. Moreover, our results showed that SCNs involved in the pathology of PD are associated

222   with multiple neurotransmitter signaling pathways, e.g. serotonin, GPCR, GABA, glutamate, and RAS.

223   **Cholinergic function in PD**

224   Genes that were highly expressed in the anterior cingulate network were significantly enriched for

225   thalamus cholinergic markers, specifically: *NPPA*, *SOSTDC1*, and *TYRP1*. These marker genes, together

226   with other markers of this cell-type, were defined based on their expression in cholinergic cells from the

227   thalamus, more specifically the hubenula (Mancarci et al. 2017). Although these genes are considered

228   thalamus-specific, according to the AHBA ontology the hubenula is not part of the thalamus. In this

229   study, most thalamus cholinergic marker genes indeed showed high expression in thalamic regions.

230   However, *NPPA*, *SOSTDC1*, and *TYRP1,* showed opposite expression patterns with mainly high

231   expression in cortical regions and low expression in limbic regions, including the thalamus. Cholinergic

232   circuits are key in cognitive functions which are impaired in neurodegenerative diseases, such as

233   Alzheimer's disease and PD (Ballinger et al. 2016). In addition, cholinergic denervation of the cortex and

234   thalamus In PD patients may contribute to the transition from PD to PD with dementia (Ballinger et al.

235   2016). We also found that glutamatergic and GABAergic marker genes were highly expressed in the

236   AHBA samples within the posterior and anterior cingulate networks, although statistical significance

237   could not be assessed due to the small number of marker genes. Interestingly, acetylcholine release by

238   cholinergic neurons affects glutamatergic and GABAergic signaling by altering the synaptic excitability

239   (Granger et al. 2015; Buendia et al. 2019). Moreover, it is thought that dysfunction of cholinergic circuits

240   contributes to cognitive decline associated with neurodegenerative diseases (Ballinger et al. 2016).

241   **NPPA, SOSTDC1, and TYRP1**

242   The cholinergic marker genes *NPPA, SOSTDC1, TYRP1* were highly expressed across the healthy donors

243   in the posterior cingulate network and anterior cingulate network compared to the other seven SCNs.

244   While the functions of these genes likely involve cholinergic signaling, several studies suggest they also

245   function as extracellular regulators of multiple other signaling pathways, including cAMP, Wnt, and β-

246   catenin signaling (Brenner et al. 1990; Hirobe 2011; Kutchko and Siltberg-Liberles 2013; De Vito 2014;

247   Bansho et al. 2017; Millan et al. 2019).

248   *NPPA* (natriuretic peptide precursor A) and other natriuretic peptides are thought to be involved in a

249   wide range of functions, including neurovascular functions, blood-brain barrier, brain homeostasis,

250   neuroprotection, and synaptic transmission by regulating the release and re-uptake of

251   neurotransmitters such as noradrenalin, dopamine and glycine (Mahinrad et al. 2016). Impaired function

252   of natriuretic peptides in brains of AD patients could accelerate neurodegeneration and may impair

253   structural integrity of the brain leading to a higher risk of cognitive decline (Mahinrad et al. 2018). Our

254   results suggest that *NPPA* might similarly be involved in PD pathogenesis given its high expression within

255   the anterior and posterior cingulate networks.

256   *SOSTDC1* (sclerostin domain-containing 1) is known as a negative regulator of bone morphogenetic

257   protein (BMP) and Wnt-signaling, but recent studies also show that *SOSTDC1* regulates natural killer cell

258   maturation and cytotoxicity (Millan et al. 2019). An increased number of natural killer cells have been

259   found in PD, but the actual relevance with PD risk is still unclear (Jiang et al. 2017). The BMP signaling

260   pathway promotes the development of midbrain dopaminergic neurons (Jovanovic et al. 2018), in which

261   *SOSTDC1* may play a role. Furthermore, *SOSTDC1* was upregulated in the striatum of Parkinsonian rats

262   that were treated by subthalamic nucleus high frequency stimulation, and is therefore suggested to

263   have neuroprotective effects (Lortet et al. 2013).

264   *TYRP1* (tyrosinase-related protein 1) produces melanocytes-specific proteins involved in the biosynthesis

265   of melanin in brain, skin and eyes (Wang and Hebert 2006; Lu et al. 2011). Melanoma and PD share

266   genes involved in the synthesis of melanin and dopamine, including *SNCA* which encodes the α-

267   synuclein protein found in Lewy bodies (Pan et al. 2012). Furthermore, neuromelanin is produced

268   almost exclusively in human catecholaminergic neurons and is responsible for the pigmentation of

269   dopaminergic neurons of the substantia nigra, and noradrenergic neurons of the locus cereleus (Pavan

270   and Dalpiaz 2017). It is considered to be protective due to its ability to chelate metals, especially iron

271   which increases with age (Pavan and Dalpiaz 2017).

272   **Disease-associations**

273   The posterior and anterior cingulate networks shared similar highly expressed genes and were likewise

274   associated with similar diseases. Both SCNs represent anatomical networks that function normally in

275   healthy brains, but their activity is reduced in aging and PD (Hafkemeijer et al. 2014; de Schipper et al.

276   2017). As part of the default mode network, both the posterior and anterior cingulate cortex have been

277   shown to be dysregulated in neuropsychiatric disorders (Broyd et al. 2009; Öngür et al. 2010). Based on

278   our analysis of transcriptomic signatures in the healthy brain, we found that the posterior cingulate

279   network showed stronger associations with memory and pain-related disorders compared to the

280   anterior cingulate networks which showed stronger associations with mental and neuropsychiatric

281   disorders. Our findings suggest that genes involved in multiple signaling pathways, such as serotonin,

282   GPCR, GABA, glutamate, and RAS, contribute to healthy functions of the posterior and anterior cingulate

283   networks.

Supplementary material

285   Supplementary material is available online.

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Notes

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422

423

424 # Tables

425 **Table 1**   Number of samples from AHBA within SCN networks.

Donors	Network								
	A	B	C	D	E	F	G	H	I
Donor 9861	72	67	157	47	74	90	26	39	83
Donor 10021	79	46	121	65	49	84	25	55	91
Donor 12876	37	24	57	28	42	45	6	17	25
Donor 14380	38	33	52	30	45	61	7	27	53
Donor 15496	34	24	41	21	39	55	13	24	69
Donor 15697	49	20	38	33	47	64	29	37	49
Total	309	214	466	224	296	399	106	199	370

A: Thalamus; B: Lateral occipital cortex, C: Posterior cingulate cortex, D: Anterior cingulate cortex, E: Temporal pole; F: Putamen; G, H, I: Cerebellum.

426

427   **Table 2** Differentially upregulated cell-type marker genes in the posterior cingulate network (C) and anterior cingulate network

428   (D). Fold-change (FC) and Benjamini-Hochberg (BH) corrected *P*-value for cell-type markers genes that were differentially

429   expressed in the PD-related networks compared to the non-PD-related networks. FC > 1 and BH < 0.05 are highlighted in red

430   text.

Gene	Marker	Network C		Network D	
		FC	BH	Estimate	BH
LHX2	Astrocyte	2.21	3.92E-03	2.00	6.46E-03
IGFBP2	Astrocyte	0.69	5.80E-02	1.18	1.78E-02
RORB	Astrocyte	0.82	3.09E-02	1.19	1.39E-02
WIF1	Bergmann	1.02	8.74E-03	1.03	7.95E-03
VIP	GabaVIPReln	1.67	4.23E-03	1.85	6.89E-03
PCSK1	Hypocretinergic	1.15	1.25E-02	1.57	1.06E-02
NEUROD6	Pyramidal	1.90	4.78E-03	1.92	6.76E-03
NPPA	ThalamusCholin	1.64	6.98E-03	2.09	6.39E-03
TYRP1	ThalamusCholin	0.81	2.41E-02	1.43	9.82E-03
SOSTDC1	ThalamusCholin	0.83	1.21E-02	1.14	6.39E-03

Fold-change (FC) and Benjamini-Hochberg (BH) corrected P-value for cell-type markers genes that were differentially expressed in the PD-related networks compared to the non-PD-related networks. FC > 1 and BH < 0.05 are highlighted in red.

431

Figure captions

Figure 1. Study overview. Transcriptomic data from AHBA were mapped to nine anatomical networks that have been defined based on healthy subjects. The posterior cingulate network and anterior cingulate network have been associated with gray matter loss in PD (PD-related), while the seven remaining networks were not related to PD (non-PD-related). We compared gene expression in each of the two PD-related networks to gene expression in all seven non-PD-related networks. Genes that were upregulated in the two PD-related networks were assessed for the overrepresentation of pathway-specific genes, cell-type marker genes, and disease-associated genes.

Figure 2. Differential expressed genes and associated pathways. Genes were analyzed for differential expression in the (A) posterior cingulate network (network C) and (B) anterior cingulate network (network D). Effect sizes were summarized across the six healthy donors of AHBA. For all genes (points) the $\log_2$ fold-change (FC; x-axis) and $-\log_{10}$ of nominal P-values (y-axis) are shown. Significant differentially expressed genes (t-test, BH-corrected $P < 0.05$ and $|FC| > 1$) are unique for each network (blue and purple points) or significant in both networks (yellow points). For each network the top 10 genes with the highest absolute FC are highlighted, which highly overlap between both networks. (C) Pathway analysis of differentially upregulated genes in the posterior cingulate network and anterior cingulate network. Both networks share similar pathways (yellow) that are hierarchically organized in the Reactome database. The posterior cingulate network showed more specific associations with pathways involved in neurotransmitter receptors and postsynaptic signal transmission (blue). The anterior cingulate network was more specifically associated with serotonin receptors (purple). See Supplementary Table 6 for gene counts and BH-corrected P-values.
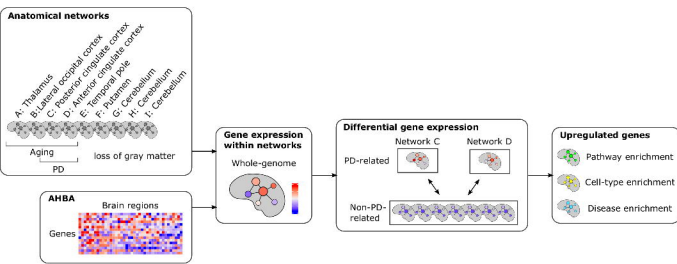
Figure 3. Expression of cell-types in anatomical networks. Gene expression was Z-scored and averaged across cell-type specific markers, samples within anatomical networks, and across the six donors in the Allen Human Brain Atlas. Separate heatmaps for each donor are shown in Supplementary Figure 1.
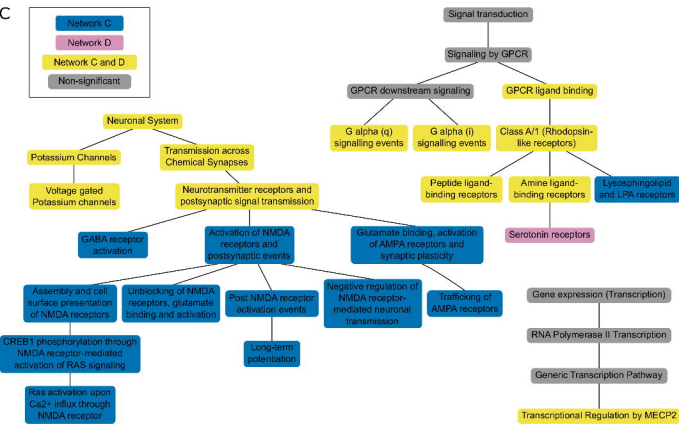
Figure 4. Expression of differentially upregulated cell-type marker genes in the posterior cingulate network (network C) and anterior cingulate network (network D). Heatmaps of differentially expressed marker genes (rows) are shown for one of the six donors in the Allen Human Brain Atlas (donor 10021). Samples from different anatomical substructures within the networks are color annotated (columns). Expression was averaged across samples from an anatomical substructure with the same acronym

457   ignoring left and right hemisphere annotations. See Supplementary Figure 2 for heatmaps for all six donors and Supplementary

458   Table 7 for full names of the region-specific acronyms.

459   **Figure 5.** Disease associations of the posterior cingulate network (network C) and anterior cingulate network (network D).

460   Differentially upregulated genes in each network were assessed for the enrichment of disease-associated genes from DisGeNET

461   (hypergeometric test, BH-corrected $P < 0.05$). Top plot shows odds ratios (ORs), and bottom plot shows significance of overlap

462   indicated with $-\log 10$ P-values (y-axis). Disorders (columns) are sorted based on highest ORs in either one of the networks.

Anatomical networks

A: Thalamus
B: Lateral occipital cortex
C: Posterior cingulate cortex
D: Anterior cingulate cortex
E: Temporal pole
F: Putamen
G: Cerebellum
H: Cerebellum
I: Cerebellum

Aging

loss of gray matter

PD

AHBA

Brain regions

Genes

Gene expression within networks

Whole-genome

Differential gene expression

Network C   Network D

PD-related

Non-PD-related

Upregulated genes

Pathway enrichment

Cell-type enrichment

Disease enrichment

Columns (left to right): Astrocyte, Basket, Bergmann, **BrainstemCholin**, CerebGranule, DentateGranule, Dopaminergic, Ependymal, ForebrainCholin, GabaPV, GabaReln, GabaReln-Calb, **GabaSSReln**, **GabaVIPReln**, **Glia**, Golgi, Hypocretinergic, Microglia, Activated microglia, Deactivated microglia, Noradrenergic, Oligo, Purkinje, **Pyramidal**, Serotonergic, SpinalCordCholinergic, Spiny, ThalamusCholin

Rows (top to bottom): Network A, Network B, Network C, **Network D**, **Network E**, Network F, Network G, Network H, Network I

Z–Score expression legend: 2, 1, 0, −1, −2