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Original Article

Detection of high expression of complex I mitochondrial genes can indicate low risk of Alzheimer's disease

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Abstract: Mitochondrial dysfunction is a consistent feature of Alzheimer's disease pathology. However, the individual involvement of over 100 mitochondrial and nuclear genes encoding respiratory chain subunits remains not clear. We aimed to assess the related genomic back ground in adult patients with Down syndrome, who typically develop Alzheimer's disease-type dementia. Selected groups of adults differing with regard to their age and cognitive status took part in our study. We used whole genome microarrays to compare the genome expression between patients with and without dementia. We found significant overexpression of the majority of mitochondrial genes (especially those encoding subunits of the complex I) in seniors remaining dementia-free despite advanced age, contrary to young adults with very early onset of dementia. Our findings suggest that detection of high activity of mitochondrial genes could indicate low susceptibility to Alzheimer's disease. The potential usefulness of expression measurement for these genes should be evaluated in general population.

Keywords: Dementia, respiratory chain, aging, neurodegenerative disorder, microarrays, mitochondrial genome

Introduction

Epidemiologic studies suggest that most people could develop Alzheimer's disease (AD) should they live long enough [1]. Although development of dementia corresponds with accumulation of amyloid β plaques and tau-protein in the brain [2, 3], mitochondrial dysfunction is a consistent feature of AD pathology. It was observed already in early-stage AD and was reported in both the brain and blood cells [4-9].

The mitochondrial genome includes 37 genes, 13 of which encode polypeptides required for the oxidative phosphorylation. However, it should be noted that the vast majority (89) of core proteins constituting the respiratory chain are of nuclear origin [10]. In addition, several genes control the processes of mitochondrial fusion, fission and the overall mitochondrial dynamics [11, 12]. Consequently, the primary genetic factor responsible for mitochondrial dysfunction in AD remains unknown.

Development of methods for prevention of AD requires identification of specific genetic profiles in individuals predisposed to AD. Surprisingly, Down syndrome (DS), which is the most common genetic cause of mental retardation, can also be regarded a "model" condition for such studies as the majority of adults with DS develop AD-type dementia in the fifth decade of life [13, 14]. Therefore, we aimed to assess the dementia-related alterations of genome expression in adults with DS. We hoped to identify molecular indicators of AD-related mitochondrial dysfunction, which could be useful in identifying of persons at high risk of AD in general population.

Materials and methods

A group of 25 selected adult patients with DS took part in our study. To assess the age-related and the AD-related alterations of genome expression we recruited patients from two homogenous age groups (37-40 years or 57-60 years). In both groups some patients presented with clear signs of dementia (hypothyroidism or depression which could mimic dementia were excluded) whereas the others remained dementia-free. Eventually, the group of younger adults with DS included seven patients without

Table 1. List of transcripts which were found significantly different (corrected p<0.05; Fold Change >2)

Older adults without dementia vs. younger adults with dementia (166 transcripts)	Upregulated: ADAMTSL4-AS1, AIF1L, APOBEC4, B4GALNT2, BIRC7, C1QTNF5, CACNA1A, CARD17, CCL15, CD200, CELF3, CENPM, CETN1, CHRNA5, CLEC1B, CLEC1B, CLEC2L, CNTNAP3B, CRYAB, EFHB, ENKUR, EPHA4, FAM183B, FAM27C, FAM27E2, FARP2, FBXW2, FKBP6, FLJ43315, FOXP4-AS1, FRMPD3, GAN, GSTA5, H2AFB1, HCG16, HGD, HPVC1, HSFX1, IL22RA2, IL24, ILF2, ITGAX, KCNE4, KCNE5, KCNK9, KRT13, KRTAP1-3, KRTAP9-9, LIF, LINC00284, LINC00376, LINC00693, LINC00880, LINC00880, LINC01013, LINC01220, LINC01271, LINC01405, LINC01506, lnc-ADAMTS7-1, lnc-ALX1-2, lnc-ASB1-1, lnc-C10orf120-1, lnc-DHX37-5, lnc-DOPEY1-1, lnc-EBF1-1, lnc-FAM150B-1, lnc-GPR26-4, lnc-JUN-6, lnc-MINA-3, lnc-PCDH18-3, lnc-PIK3CG-2, lnc-PITRM1-2, lnc-PPP1R26-1, lnc-RASGRP1-4, lnc-RFPL4B-4, lnc-RREB1-4, lnc-SETD6-3, lnc-STIM2-2, lnc-STX18-2, lnc-TMEM72-1, lnc-TSC22D1-1, lnc-TSHZ1-1, lnc-TSPYL5-2, lnc-TTC27-1, lnc-TTC40-3, lnc-VGLL3-3, lnc-XRCC4-1, LOC100128494, LOC100130193, LOC100130370, LOC100130872, LOC100132287, LOC100287808, LOC100507073, LOC100507191, LOC101926940, LOC101928424, LOC101928475, LOC101928565, LOC101928670, LOC101929572, LOC727993, LOC93432, LY6G6C, MEG3, NEDD4L, NEURL1, NRXN1, OR2L2, PAFAH1B2, PF4V1, PLA1A, PRR14L, PRR7, PRSS1, RAB3B, RBMX2, RNU2-1, RNVU1-18, RPL23AP7, RPPH1, RPS6KL1, RXRG, SBK3, SCARNA14, SEMG2, SERPINA10, SLED1, SMG7, SNORA16A, SNORA16B, SNORA21, SNORA45B, SNORD37, SNORD46, SNORD68, SNORD83B, SNORD9, SP2, SPRY1, SPRY4, SYT14, THRB, TM4SF4, TMEM240, TMEM59, TMPRSS9, XLOC_l2_000900, XLOC_l2_004640, XLOC_l2_005691, XLOC_l2_008352, XLOC_l2_010328, ZG16, ZNF138, ZNF154, ZNF542P, ZNF713, Mitochondrial genes: ATP8, CYTB, ND1, ND2, ND3, ND4, ND5 Downregulated: MST1R,
Older adults without dementia vs. younger adults without dementia (24 transcripts)	Upregulated: BNC2, DKFZp434J0226, DRD5, FAM150B, FAM71F2, IL36B, ISL2, KHK, LINC00539, lnc-C15orf2-1, lnc-CDH18-4, lnc-DMRTA1-5, lnc-IRF2BPL-2, lnc-PROP1-3, LOC100507420, LOC388210, LRRC70, MYZAP, NUDCD1, SNORA45A, TBC1D3C, WDR5 Downregulated: ANXA8L1, S100A16,
Older adults without dementia vs. older adults with dementia (4 transcripts)	Upregulated: MYZAP, TACSTD2, PF4V1, DDX11L16
Older adults with dementia vs. younger adults with dementia (1 transcript)	Upregulated: LRRD1
Older adults with dementia vs. younger adults without dementia (0 transcripts)	-
Younger adults with dementia vs. younger adults without dementia (1 transcript)	Donwregulated: SEMA3B-AS1
Older adults vs. younger adults (1 transcript)	Upregulated: lnc-MINA-3

dementia and six with dementia, and the older group with DS consisted of five patients without dementia and seven with dementia. All patients or their legal representatives signed informed consent forms to participate in the study. The Jagiellonian University Ethics Committee accepted the study protocol.

We assessed the genome expression in blood mononuclear cells. Total RNA was extracted from blood samples with use of PAX gene system (PreAnalytix). Next, we used whole genome microarrays (Sure Print Human Gene Expression 8x60K v2 Microarrays; Agilent) to compare genome expression between all subgroups of patients. Sure Print G3 Human Gene Expression Microarrays provide a comprehensive coverage of entire human genome (50.599 genes and transcripts) with high specificity of target detection and very wide dynamic range allowing for detection of biological features with very high and with very low expression.

Microarray data analysis was performed with use of Gene Spring Software (www.genespring.com) and by means of DAVID Bioinformatics Resources 6.7 [15]. Single color expression technology dedicated for Agilent microarrays was implemented. Raw data were initially normalized to the signals of two control genes (*B2M* and *GAPDH*), which are routinely used as reference-genes in assessment of gene expression. The moderated t-test was applied with the Westfall-Young permutative correction for multiple testing. A corrected value of $P<0.05$ was considered significant.

The primary microarray data were submitted to the GEO public repository and are accessible through GEO Series accessionnumber

GSE63870 [http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63870].

Results

The initial comparison of younger and older patients did not reveal significant differences with regard to expression of genes related to mitochondrial function. On contrary, the comparison of younger adults diagnosed with early dementia and of older adults with good cognitive status (apparently less susceptible to AD) revealed 166 transcripts. The genomic pathway analysis showed significant enrichment (P=0.001) for genes representing the mitochondrial oxidative phosphorylation. Seven mitochondrial genes (ND1, ND2, ND3, ND4, ND5, CYTB, ATP8) encoding the first, third and fifth respiratory chain complexes proved significantly upregulated in seniors without dementia.

The remaining groups of patients did not differ with regard to genes encoding proteins involved in oxidative phosphorylation or nuclear genes known as regulators of mitochondrial function.

Table 1 shows details of the genome expression differences between the tested groups.

Discussion

As we did not find any significant age-related alteration of genome expression, the observed differences between younger adults with dementia and older adults without dementia probably reflect the pathology of AD. Although the tested cohort was relatively small, the subgroups of patients were homogenous and we detected strong statistical significance with regard to the expression of transcripts representing mitochondrial complexes I, III and V. This supports the "mitochondrial" hypothesis of AD [5, 6]. It should be noted, that the very early development of dementia at the end of the fourth decade of life as well as lack of dementia at the end of the sixth decade of life is uncommon in adults with DS (typical life expectancy reaches in them 60 years) [16, 17]. Consequently, young adults with dementia and seniors without dementia should have specific expression profiles of genes related to pathology of AD.

We detected overexpression of the majority of complex I subunits (five out of seven transcripts) in seniors without dementia, what suggests the importance of dysfunction of this complex in AD pathology. Our observations correspond with previous reports on the typically decreased activity of complex I in brain tissue in DS [19].

On contrary, we did not detect any significant differences with regard to nuclear genes encoding the majority of mitochondrial proteins or to genes known to regulate mitochondrial function. Therefore, it could be postulated that dysfunction of mitochondrial complex I genesis a primary phenomenon in AD. However, it is not clear if the overexpression of mitochondrial *CYTB* and *ATP8* genes could be secondary to the hyperactivity of complex I, as deficits in activity of complexes III and V were also reported in the past [19].

We did not detect any expression variations with regard to genes regulating mitochondrial function. However, biochemical impairment of oxidative phosphorylation by amyloid β plaques and tau-protein was reported in an animal model [20, 21], what confirms the complexity of AD pathology on the genome and proteome levels. Nevertheless, in our opinion, further studies are necessary to confirm the potential of mitochondrial gene expression measurement for early detection of persons at increased risk for development of AD in general population.

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Disclosure of conflict of interest

None.

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