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Dysfunctions of mitochondria in close association with strong perturbation of long noncoding RNAs expression in down syndrome

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ABSTRACT

Trisomy 21 is the most common chromosomal disorder and underlies Down syndrome. Epigenetics, such as DNA methylation and post-translational histone modifications, plays a vital role in Down syndrome. However, the functions of epigenetics-related long noncoding RNAs (lncRNAs), found to have an impact on neural diseases such as Alzheimer's disease, remain unknown in Down syndrome. In this study, we analyzed the RNA sequencing data from Down syndrome-induced pluripotent stem cells (iPSCs) and normal iPSCs. A large number of lncRNAs were identified differentially expressed in Down syndrome-iPSCs. Notably, stronger perturbation was shown in the expression of lncRNAs compared to protein coding genes (Kolmogorov–Smirnov test, $P < 0.05$), suggesting that lncRNAs play more important roles in Down syndrome. Through gene set enrichment analysis and bi-clustering, we also found that most of the differential expressed lncRNAs were closely associated with mitochondrial functions (e.g. mitochondrion organization, $P = 3.21 \times 10^{-17}$; mitochondrial ATP synthesis coupled electron transport, $P = 1.73 \times 10^{-19}$ and mitochondrial membrane organization, $P = 4.04 \times 10^{-8}$). PCR-array and qRT-PCR results revealed that almost all genes related to mitochondria were down-regulated in Down syndrome-iPSCs, implying that mitochondria were dysfunctional in Down syndrome (e.g. *ATP5B*, Fold Change = −8.2317; *COX6A1*, Fold Change = −12.7788 and *SLC25A17*, Fold Change = −22.1296). All in all, our study indicated that a stronger perturbation of lncRNAs expression may lead to the dysfunction of mitochondria in Down syndrome.

1. Introduction

With an incidence of approximately 1 in 700–1000 live births, Down Syndrome (DS), or trisomy 21 (triplication of chromosome 21), is the most common genetic disorder and the leading genetic cause of intellectual disability in humans (El Hajj et al., 2016).Theoretically, this would lead to a 1.5-fold increase in the abundance of RNAs transcribed from chromosome 21, but many studies have demonstrated that the levels of many of these RNAs deviate from this theoretical increase. Therefore, it is necessary to explore the underlying cause of this observed variation in gene expression that would be expected in DS. Conceivably, epigenetic mechanisms may play a role in regulating gene expression in trisomy 21 chromosomes (Dekker et al., 2014).

An epigenetic change is defined as a stably heritable phenotype that results from chromosomal changes that do not involve alterations in DNA sequence (Berger et al., 2009). The rapid growth of epigenomic research and data from high-throughput approaches has opened up novel therapeutic options for several diseases, including neurological and genetic disorders. Evidently, learning and memory, which are both impaired in many individuals with DS, can be modulated by epigenetic mechanisms (Dekker et al., 2014). Generally, epigenetic changes occur through five main remodeling processes: DNA methylation, post-translational histone modifications, nucleosomal positioning, histone variant incorporation, and the action of small noncoding RNAs and long noncoding RNAs (lncRNAs) (Weber et al., 2015). Some of these mechanisms contribute to the etiology of DS, such as DNA methylation and histone modifications.

Increasing evidence suggests has suggested that noncoding RNAs may also play important roles in DS. For example, the five microRNAs miRNA-99a, miRNA-125b-2, miRNA-155, miRNA-802, and let-7c are located on human chromosome 21 and therefore are likely over-expressed in DS (Sanchez-Mut et al., 2012). LncRNAs, which by definition are noncoding RNAs of ≥200 nt, have also been demonstrated to contribute to neurological diseases. For instance, the lncRNAs BACE1-

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Table 1
Top 10 Up- or Down- regulated protein coding genes.

Gene_ID	locus	DS-iPSC (FPKM)	Normal-iPSC (FPKM)	Regulation	q_value
BRINP3	chr1:190066796-190463427	3.6668	0	Up	0.000625
EN2	chr7:155250823-155257526	30.9068	0	Up	0.000625
GALP	chr19:56687388-56697144	7.59706	0	Up	0.000625
HOXA2	chr7:27139972-27142394	5.12229	0	Up	0.000625
ZNF804A	chr2:185460943-185804214	2.00123	0	Up	0.000625
POU3F2	chr6:99282579-99286666	24.8242	0.070946	Up	0.041454
NR2F1	chr5:92745064-92930317	32.75	0.258716	Up	0.000625
RFX4	chr12:106976684-107156582	8.91445	0.073607	Up	0.049514
SP5	chr2:171034654-171574498	22.4772	0.200757	Up	0.045578
A2M	chr12:9217772-9268657	74.1354	0.849891	Up	0.000625
CCL28	chr5:43381599-43412488	0	3.60053	Down	0.000625
DPPA5	chr6:74062784-74063999	0	3.04267	Down	0.000625
IZUMO2	chr19:50655804-50666538	0	1.78359	Down	0.000625
MT1HL1	chr1:237164676-237167718	0	8.19894	Down	0.000625
PIWIL2	chr8:22132809-22213584	0.196772	3.52174	Down	0.000625
NAPRT1	chr8:144655659-144660521	0.777804	14.0963	Down	0.000625
HRH3	chr20:60790016-60795323	0.383825	7.08107	Down	0.000625
ZFP28	chr19:57050316-57078833	0.118095	2.26774	Down	0.025598
PCDHB15	chr5:140624916-140627801	0.180974	3.50274	Down	0.002559
ZNF506	chr19:19867180-20008579	0.374701	7.82492	Down	0.014309

Table 2
Top 10 Up- or Down- regulated lncRNAs.

Gene_ID	locus	DS-iPSC (FPKM)	Normal-iPSC (FPKM)	Regulation	q_value
TCONS_00125451	chr4:171045946-171128422	47.4958	0	Up	0.000625
NONHSAG036455	chr3:154958731-155011965	39.7049	0	Up	0.000625
TCONS_00114814	chr3:39621246-39844662	39.0006	0	Up	0.03363
NONHSAG033142	chr22:16147978-16193004	38.5451	0	Up	0.000625
TCONS_00113274	chr3:39621246-39844662	37.6061	0	Up	0.000625
TCONS_00010849	chr1:188783595-188791438	24.4225	0	Up	0.000625
TCONS_00169801	chrX:148448021-148460806	17.5369	0	Up	0.000625
TCONS_00013888	chr1:232497241-232505193	17.3107	0	Up	0.012749
TCONS_00122548	chr4:131045291-131064737	14.3599	0	Up	0.01837
NONHSAG039424	chr4:180310287-180386459	13.2204	0	Up	0.000625
NONHSAG025332	chr19:23115570-23128510	0	8.64471	Down	0.001171
TCONS_00135778	chr6:120326758-120449768	0	8.66443	Down	0.002123
TCONS_00142387	chr7:51384839-51385959	0	9.14746	Down	0.000625
TCONS_00045501	chr14:41116980-41211591	0	13.515	Down	0.000625
NONHSAG026475	chr19:54292035-54292422	0	14.2561	Down	0.000625
TCONS_00109427	chr3:80569662-80683125	0	16.2134	Down	0.049924
TCONS_00164843	chrX:114929994-114943246	0	32.4565	Down	0.000625
TCONS_00150712	chr8:76687676-76823785	0	34.2599	Down	0.000625
NONHSAG013617	chr13:56865457-57309621	0	49.3447	Down	0.000625
NONHSAG018021	chr15:97404717-97433527	0	85.7737	Down	0.000625

AS and BC200 in Alzheimer's disease (AD), BDNF-AS in Huntington's disease, and naPINK1 in Parkinson's disease (PD) (Qureshi et al., 2010). Because DS displays certain characteristics of a neurological disease and some noncoding RNAs have been found to be differentially expressed in DS, it is reasonable to suspect that lncRNAs may also play roles in DS. However, the involvement of lncRNAs in DS remains unknown. Therefore, we investigated whether lncRNAs contribute to the etiology of DS.

2. Materials and methods

2.1. RNA sequencing dataset

The RNA sequencing dataset GSE52249 was downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). It contains seven transcriptome profiles for induced pluripotent stem cells (iPSCs; four euploid and three trisomy 21) that were derived from monozygotic twins (Hibaoui et al., 2014).

2.2. Analysis of differentially expressed genes in DS

Reads were aligned to the human genome (reference genomehg19) using Botwie2. Tophat is used for transcriptomic reads alignment against the reference genome and splice junctions identification based on the alignment (Kim et al., 2013). The aligned reads were then assembled into transcripts using Cufflinks version 1.0.3 with default parameters (Trapnell et al., 2012). The Cuffdiff program was used to conduct differential expression tests between iPSC samples from DS individuals (DS-iPSC) and euploid individuals (Normal-iPSC) (FDR-adjusted p-value < 0.05).

2.3. Gene set enrichment analysis and clustering of functional terms

Gene set enrichment analysis was performed to analyze the functions of differentially expressed lncRNAs. Briefly, each lncRNA was used as a profile to compute the Pearson correlation for each protein-coding gene and then rank those genes based on their correlation coefficients. This rank was used to identify gene sets containing genes that were differentially expressed in DS-iPSCs. An association matrix between lncRNA loci and gene sets was constructed using a false-