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Expression of trisomic proteins in Down syndrome model systems

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ABSTRACT

Down syndrome (DS) is the most common genetic aberration leading to intellectual disability. DS results from an extra copy of the long arm of human chromosome 21 (HSA21) and the increased expression of trisomic genes due to gene dosage. While expression in DS and DS models has been studied extensively at the RNA level, much less is known about expression of trisomic genes at the protein level. We have used quantitative Western blotting with antibodies to 20 proteins encoded by HSA21 to assess trisomic protein expression in lymphoblastoid cell lines (LCLs) from patients with DS and in brains from two mouse models of DS. These antibodies have recently become available and the 20 proteins largely have not been investigated previously for their potential contributions to the phenotypic features of DS. Twelve proteins had detectable expression in LCLs and three, CCT8, MX1 and PWP2, showed elevated levels in LCLs derived from patients with DS compared with controls. Antibodies against 15 proteins detected bands of appropriate sizes in lysates from mouse brain cortex. Genes for 12 of these proteins are trisomic in the Tc1 mouse model of DS, but only SIM2 and ZNF295 showed elevated expression in Tc1 cortex when compared with controls. Genes for eight of the 15 proteins are trisomic in the Ts65Dn mouse model of DS, but only ZNF294 was over expressed in cortex. Comparison of trisomic gene expression at the protein level with previous reports at the mRNA level showed many inconsistencies. These may be caused by natural inter-individual variability, differences in the age of mice analyzed, or post-transcriptional regulation of gene dosage effects. These antibodies provide resources for further investigation of the molecular basis of intellectual disability in DS.

1. Introduction

Approximately 1/750 newborns in the United States and 1/1000 in Europe are diagnosed with Down syndrome (DS) (CDC, 2006; Irving et al., 2008), which is caused by an extra copy of all or part of the long arm of human chromosome 21 (HSA21). The phenotype of DS is both complex, affecting most organs and organ systems, and variable in severity. Common to all individuals with DS are characteristic facial features, short stature, hypotonia and some level of intellectual disability (ID). Neurological abnormalities have a strong impact on the quality of life of the individuals with DS because, in addition to ID, they also include increased risks of seizures, autism and depression, the early development of Alzheimer's disease (AD) pathology, and early onset AD-like dementia (Henderson et al., 2007; Lott and Dierssen, 2010; Wiseman et al., 2009).

The DS phenotype is hypothesized to result from perturbations caused by increased transcription from trisomic genes due to dosage. HSA21q encodes 161 classical protein coding genes, 5 microRNAs, and >350 additional genes and experimentally supported gene models that cannot be unambiguously classified as either protein coding, functional RNA or transcriptional noise (Sturgeon and Gardiner, 2011). A focus on protein coding genes reveals a plethora of intriguing functional properties (Sturgeon et al., 2012) and, for the majority of genes, it is not hard to propose mechanisms by which they reasonably may underlie or contribute to one or more neurological phenotypic features. One way to narrow the field of candidate genes is to focus on those genes that are indeed over expressed in trisomy. This approach has led to numerous mRNA expression studies using microarrays and quantitative RT-PCR with RNA derived from fetal brains, amniocytes, neurospheres and cell lines from DS, and tissues and cells from mouse models of DS. The overall conclusion from such experiments is that trisomic genes are, on average, over expressed by approximately 50%, but the situation is complicated by gene-specific differences related to tissue, developmental timing and/or age, and by variation in expression level among individuals (summarized in Vilardell et al., 2011). Much less has been reported on trisomic gene expression at the protein level, with typical reports assessing only 3–5 proteins (Cheon et al., 2003, 2008; Choi et al., 2009; Siddiqui

Abbreviations: DS, Down syndrome; HSA21, Human chromosome 21; ID, Intellectual disability; LCL, Lymphoblastoid cell line.

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et al., 2008). In part, this lack of information is due to a lack of quality antibodies for standard measurement of protein levels by Western blotting. Given the poor correspondence between mRNA levels and protein levels (Maier et al., 2009; Schwanhäusser et al., 2011), however, it remains of interest to know which HSA21 genes are indeed over expressed at the protein level. To contribute to this effort, we have used Western blots to validate antibodies that have recently become available for 20 HSA21 proteins that largely have not been well characterized for potential roles in DS. We have assessed expression of these 20 proteins in DS-derived lymphoblastoid cell lines and cortex obtained from the Ts65Dn and Tc1 mouse models of DS and compared gene dosage effects at the protein level with previous reports of gene dosage effects at the mRNA level.

2. Materials and methods

2.1. Lymphoblastoid cell lines (LCLs)

Cell lines, 3 control (AG18210, AG18256 and AG19420) and 3 DS (AG09802, AG10316 and GM04927), were purchased from Coriell Cell Repositories (Camden, NJ, USA). All cell lines were derived from individuals between the ages of 24 and 41, and with the exception of AG18256, all were males. Cells were grown under standard conditions as recommended by the supplier in DMEM supplemented with bovine serum and antibiotics. Cells were harvested approaching confluence and protein lysates prepared in IEF buffer (8 M urea, 4% CHAPS, 50 mM Tris).

2.2. Mouse brain tissue

Brains, snap frozen in liquid nitrogen, from Tc1 and littermate controls (males, aged 7–8 months) were a generous gift from EM Fisher, FK Wiseman (University College London Institute of Neurology) and V Tybulewicz (MRC National Institute for Medical Research, London, UK). Brains were heat stabilized in the Stabilizor T1 (Denator AB) as described (Ahmed and Gardiner, 2011; Ahmed et al., 2012) and cortex dissected. Cortex samples from Ts65Dn and littermate controls (males, 7–8 months old) were a subset of the same samples as used in Ahmed et al. (2012). Whole tissue protein lysates were prepared in IEF buffer as described (Ahmed and Gardiner, 2011).

2.3. Primary antibodies

Primary antibodies raised against 20 HSA21 proteins have recently become available (Aviva Systems Biology, San Diego, CA, USA). Information for these antibodies is provided in Table 1. Primary antibodies against six well-studied HSA21 proteins were purchased as follows: APP (Cell Signaling Technology, MA, USA); TIAM1 (Abcam Inc. Cambridge, MA, USA); SOD1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA); DYRK1A (Abnova, Taiwan); and ITSN1 (BD Biosciences, CA, USA), and RCAN1 (Sigma, USA).

2.4. Western blotting

Twenty micrograms of protein lysates (previously determined by standard methods to be within the linear range of antibody detection) per lane were separated at 170 V, constant voltage for 2 ½ hours on SDS-PAGE gels (8% or 10% depending upon size the proteins to be detected). Following electrophoresis, proteins were transferred to PVDF membranes at 250 mA constant current for 1 ½ hours. Membranes were blocked with 5% (w/v) non-fat dry milk in TBST (Tris-buffered saline, 0.1% Tween 20), followed by overnight incubation at 4 °C with primary antibody. Detection of bound primary antibodies was performed with alkaline-phosphatase-conjugated goat anti-rabbit (Invitrogen, CA, USA) or goat anti-mouse (Cell Signaling Technology, MA, USA) secondary antibodies. Signals were detected with CDP-Star Chemiluminescence reagent; imaging and quantitation were carried out using the Diana III CCD camera and Aida software (Raytest, Inc, Germany). All membranes were stripped in 0.2 M Glycine pH 2.5/0.05% Tween 20, at 70 °C for 20 min, and re-probed with actin antibody (Sigma, USA). Protein expression in trisomy vs. controls was determined from replicate gels. When no signals from an antibody were detected with 20 μg of protein lysate, amounts per lane were increased to 40 or 60 μg.

Table 1
Antibodies to HSA21 encoded proteins.

Protein	Accession no.	Catalogue no.	Dilution
ZNF294 (LTN1)	NP_056380	ARP43201_P050	1:500
CCT8	NP_006576	ARP45837_P050	1:1000
C21ORF59	NP_067077	ARP45873_P050	1:500
IFNGR2	NP_005525	ARP46611_P050	1:500
DONSON	NP_060083	ARP45862_P050	1:1500
CHAF1B	NP_005432	ARP45826_P050	1:1000
SIM2	NP_005060	ARP38551_P050	1:500
LCASL (C21ORF13)	NP_689718	ARP45912_P050	1:1000
SH3BGR	NP_031367	ARP45764_P050	1:1000
MX1	NP_002453	ARP46077_P050	1:500
ZNF295	NP_065778	ARP32731_P050	1:500
TMPRSS3	NP_115781	ARP57684_P050	1:1000
NDUFV3	NP_066553	ARP45758_P050	1:500
CBS	NP_000062	ARP45746_P050	1:1000
RRP1	NP_003674	ARP45812_P050	1:2000
PWP2	NP_005040	ARP45822_P050	1:500
PFKL	NP_002617	ARP45774_T100	1:500
ADARB1	NP_001103	ARP40342_T100	1:2000
FTCD	NP_006648	ARP41577_P050	1:1000
PRMT2	NP_001526	ARP40196_T100	1:1500

Proteins are listed in order from centromere to telomere.

2.5. Statistical analysis

For each antibody, signals were normalized to actin. Average values from replicate experiments were used in statistical analysis using the unpaired Student *t*-test (GraphPad Prizm). $p<0.05$ was considered statistically significant.

2.6. Peptide blocking

Blocking peptides for selected antibodies were purchased from Aviva Systems Biology (San Diego, CA, USA). Sequences are included in Table 2. Peptides were incubated at a final concentration of 1 μg/ml together with primary antibody in 5% (w/v) non-fat dry milk in TBST for 1 hour at room temperature. Western blot membranes, prepared from parallel gel lanes containing either control LCL lysates or control mouse cortex lysates, were incubated with primary antibody ± peptide at 4 °C overnight. Secondary antibody treatments, washes and signal detection were carried out as described above for Western blotting.

3. Results

Intellectual disability is a critical phenotypic feature impacting quality of life for people with DS. Understanding protein expression in brain is, therefore, particularly important. However, because of the inherent challenges in controlling post mortem intervals and conditions, and the lability of protein profiles, obtaining human brain samples that reliably reflect the in vivo molecular state is difficult. To assess human protein expression, we therefore chose to use LCLs derived from controls and individuals with DS. LCLs also provide a renewable resource, numerous samples are available, and they have been used in DS RNA expression experiments. Brain expression in trisomy was evaluated using cortex from two popular mouse models of DS, the Ts65Dn and the Tc1 (reviewed in Roubertoux and Carlier, 2010). The Ts65Dn is trisomic for a segment of mouse chromosome 16 that carries orthologs of 88 of the 161 HSA21 protein coding genes (Sturgeon and Gardiner, 2011). The Tc1model carries a human chromosome 21 in a background of the normal