

# BDNF and DYRK1A Are Variable and Inversely Correlated in Lymphoblastoid Cell Lines from Down Syndrome Patients

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**Abstract** Down syndrome or trisomy 21 is the most common genetic disorder leading to mental retardation. One feature is impaired short- and long-term spatial memory, which has been linked to altered brain-derived neurotrophic factor (BDNF) levels. Mouse models of Down syndrome have been used to assess neurotrophin levels, and reduced BDNF has been demonstrated in brains of adult transgenic mice overexpressing *Dyrk1a*, a candidate gene for Down

syndrome phenotypes. Given the link between DYRK1A overexpression and BDNF reduction in mice, we sought to assess a similar association in humans with Down syndrome. To determine the effect of DYRK1A overexpression on BDNF in the genomic context of both complete trisomy 21 and partial trisomy 21, we used lymphoblastoid cell lines from patients with complete aneuploidy of human chromosome 21 (three copies of *DYRK1A*) and from patients with

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partial aneuploidy having either two or three copies of *DYRK1A*. Decreased BDNF levels were found in lymphoblastoid cell lines from individuals with complete aneuploidy as well as those with partial aneuploidies conferring three *DYRK1A* alleles. In contrast, lymphoblastoid cell lines from individuals with partial trisomy 21 having only two *DYRK1A* copies displayed increased BDNF levels. A negative correlation was also detected between BDNF and *DYRK1A* levels in lymphoblastoid cell lines with complete aneuploidy of human chromosome 21. This finding indicates an upward regulatory role of *DYRK1A* expression on BDNF levels in lymphoblastoid cell lines and emphasizes the role of genetic variants associated with psychiatric disorders.

**Keywords** Down syndrome · *DYRK1A* · BDNF · Lymphoblastoid cell lines · mRNA · Protein

### Abbreviations

BDNF Brain-derived neurotrophic factor  
DS Down syndrome  
LCLs Lymphoblastoid cell lines

### Introduction

Down syndrome (DS), resulting from partial or complete trisomy for chromosome 21, is the most common genetic cause of mental retardation [1]. Individuals with DS exhibit multiple cognitive deficiencies, including impaired short- and long-term spatial memory [2]. Such cognitive deficiencies can be traced to abnormalities in the survival and functional maintenance of neurons, processes which are dependent upon neurotrophic factors. One of these growth factors, brain-derived neurotrophic factor (BDNF), regulates neuronal cell survival, differentiation, synaptic plasticity, and neurogenesis. BDNF has also been implicated in processes required for learning and memory, such as long-term potentiation [3]. Importantly, abnormal BDNF levels in the brain are associated with a wide range of neurodegenerative

diseases, such as Alzheimer's disease, Parkinson's disease, and DS [4].

Mouse models of DS have been critical in developing our understanding of the expression of neurotrophins in conditions of cognitive impairment. For example, reduced levels of BDNF have been detected in the hippocampus and cortex of one commonly used mouse model of DS, Ts65Dn [5, 6]. Similarly, reduced BDNF expression has also been demonstrated at the mRNA level [7]. In another model, transgenic mice overexpressing *Dyrk1a* (orthologous to the gene found on human chromosome 21), BDNF protein expression is significantly reduced in adult brains [8]. Further, the altered expression of BDNF can be corrected by supplementing the drinking water of mice overexpressing *DYRK1A* with green tea polyphenol extract, which contains epigallocatechin gallate, an inhibitor of *DYRK1A* [9]. *DYRK1A* has been proposed as a candidate gene for DS phenotypes [10]. Transgenic mice overexpressing *DYRK1A* exhibit impaired spatial learning and memory, suggesting that *DYRK1A* overexpression in DS may contribute to mental retardation [11]. Genetic association tests across five major personality factors point to several SNPs within genes known for their functions in the brain and their effects on behavior and mental disorders. They notably include BDNF and *DYRK1A*, and these findings seem to reflect the phenotypic links between personality and psychiatric disorders [12]. We aimed to assess variability of *DYRK1A* and BDNF levels and their association in healthy individuals and DS patients to determine the link between these proteins and cognitive impairment.

### Methods and Materials

#### Subjects and Sample Description

Blood samples were collected from participants into heparinized containers, and containers were immediately placed on ice until processed. Plasma was obtained by centrifugation of heparinized containers for 15 min at 2,000×g at 4 °C, then rapidly frozen and stored at –80 °C until analysis. Epstein–Barr virus-transformed lymphoblastoid cell lines (LCLs) were derived from B lymphocytes of healthy individuals and unrelated individuals with DS. LCLs are easy to grow and are widely used to study genotype–phenotype correlation [13]. Parents of patients from the Institut Jérôme Lejeune gave their informed consent, and the French biomedical ethics committee gave its approval for this study (Comité de Protection des Personnes dans la Recherche Biomédicale number 03025). Written informed consent was obtained from the participants or from their families by the cytogenetic service of the Hôpital Necker Enfants Malades and Hôpital Saint Etienne, the University of

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Geneva, the University of Adelaide, the University of Nijmegen, and the University of Ghent. Cell lines from control individuals were also obtained with their written informed consent. LCLs were cultured in Opti-MEM with GlutaMax (Invitrogen, Cergy, France) supplemented with 5 % fetal bovine serum from a unique batch and 1 % penicillin and streptomycin mix (10,000 U/ml). Cell lines were grown at 37 °C in humidified incubators under 5 % CO<sub>2</sub>. For detailed structural Hsa21 aberration detection, high-resolution array CGH with NimbleGen HG18 chromosome 21 specific 385K arrays was used (B3752001-00-01; Roche NimbleGen Systems, Madison, WI, USA). The 385K average probe distance was 70 bases.

#### mRNA Expression Data

Total RNAs were obtained from LCLs using NucleoSpin RNA II kit in accordance with the manufacturer's protocol (Macherey Nagel, France). Using human pangenomic Illumina microarrays containing 48,701 probes, expression profiles for mRNAs from 43 LCLs from individuals with DS were obtained. Among the 48,701 probes on the microarrays, 11,224 probes corresponding to 9,758 genes displayed significant expression in LCLs. Results from three independent experiments (43 total samples from people with DS) were analyzed to identify differentially expressed genes. In a second experiment, Illumina bead-chips were used to analyze 24 healthy individuals. Data were normalized using quantile normalization. Microarray data have been deposited in the GEO database with the number GSE34459.

#### Protein Preparation

Cells were harvested by centrifugation, washed in 5 ml PBS, centrifuged again, and stored at –80 °C. Cell lysates were obtained from 10 to 20 × 10<sup>6</sup> cell pellets treated with 300 μl of lysis buffer [Tris, 50 mM, pH8; NaCl, 150 mM; Igepal, 1 % (Sigma-Aldrich, France); SDS, 0.1 %] containing protease inhibitors (1 mM Pefabloc SC, 5 μg/ml E64, and 2.5 μg/ml Leupeptin). After centrifugation for 10 min at 15,000 × g at 4 °C, the cell lysate was stored at –80 °C. The protein content of lysates was measured by Bio-Rad Protein Assay reagent (Bio-Rad).

#### BDNF Protein Analysis

BDNF levels were measured in either 100 μl of plasma (twice-diluted) or cell lysates from LCLs using the BDNF E-Max Immunoassay (ELISA E-Max, Promega, Madison, WI, USA). Plasma or cell lysates were incubated on a 96-well polystyrene ELISA plate previously coated with anti-BDNF monoclonal antibody. A standard curve was generated from

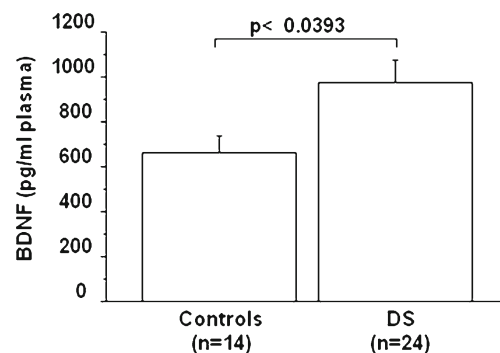
serial dilutions of a human recombinant BDNF solution at 1 μg/ml. The captured neurotrophin was bound by a second specific antihuman BDNF polyclonal antibody, which was detected using a species-specific antibody conjugated to horseradish peroxidase (HRP). After removal of unbound conjugates, bound enzyme activity was assessed by chromogenic substrate for measurement at 450 nm by a microplate reader (Flex Station3, Molecular Device). All assays were performed in duplicate.

#### DYRK1A Protein Analysis

Protein preparation was blotted on a nitrocellulose membrane (Protran<sup>R</sup>) using a slot blot apparatus (Proteigene, France). After transfer, membranes were saturated by incubation in 5 % w/v nonfat milk powder in Tris–saline buffer (1.5 mM Tris base, pH 8; 5 mM NaCl; 0.1 % Tween-20) and incubated overnight at 4 °C with antibodies against DYRK1A (1/250; Abnova Corporation, Tebu, France). Binding of the primary antibody was detected by incubation with HRP-conjugated secondary antibody using the Western Blotting Luminol Reagent (Santa Cruz Biotechnology, Tebu, France). Ponceau-S coloration was used as an internal control. Digitized images of the immunoblots obtained using an LAS-3000 imaging system (Fuji Photo Film Co., Ltd.) were used for densitometric measurements with an image analyzer (UnScan It software, Silk Scientific Inc.). All assays were performed in duplicate.

#### Statistical Analysis

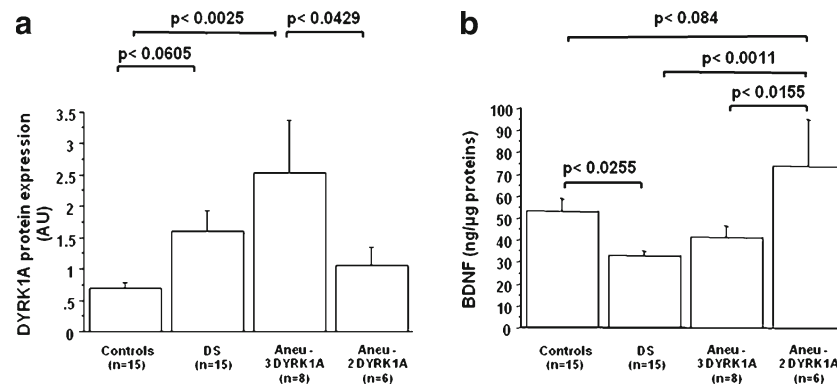
Results are expressed as mean ± SEM. Statistical analysis was performed with one-way ANOVA followed by Fisher post hoc test or by Student's unpaired *t* test using Statview software. Correlations were assessed with nonparametric Spearman's rank correlation test. Data were considered significant when  $p \leq 0.05$ . A  $p$  value of 0.06–0.10 was considered to indicate a strong statistical tendency due to the small sample size.



**Fig. 1** Plasma BDNF protein levels in individuals with DS. BDNF levels were measured in plasma samples from 24 individuals with DS (DS) and 14 healthy participants (Controls). Student's unpaired *t* test was used for statistical analysis

**Table 1** HSA 21 aberrations

Patient ID	Initial findings	Type of aberration	Aberration 1		Aberration 2		Aberration 3		Aberration 4		Aberration size (Mb)	Likely copy number of DYRK1A (chr21:37714471-37809549)
			Start (bp)	End (bp)	Start (bp)	End (bp)	Start (bp)	End (bp)	Start (bp)	End (bp)		
JDE	dup from 31.95/35.08 Mb to telomere	dup, dup	34,343,750	43,706,250	44,896,250	46,422,250	–	–	–	–	10,889	3
IGU	dup21 dup between 36.36/36.43 Mb and bk2 41.75/42.66	dup, dup	18,982,250	18,999,750	36,426,250	42,589,750	–	–	–	–	6,181	3
SDU	46,XX, dup(21) (pter->q13::q21.3->qter)	dup	21,047,250	qter	–	–	–	–	–	–	25,898	3
40446	47,XX,+der(21)	dup, trip, dup, dup	14,236,250	14,400,750	14,404,250	17,148,250	17,151,750	17,585,750	36,807,750	39,152,750	5,688	3
NBA	46, X, der (X)(X;21) (p22.2;q21.3)	del	cen	31,774,750	–	–	–	–	–	–	22,055	3
ASP	Pericentric inversion and a minute chromosome											
45895	47,XY,+der(21)	dup	28,390,250	qter	–	–	–	–	–	–	18,555	3
43131	21q22dup	dup	35,876,750	40,685,750	–	–	–	–	–	–	4,809	3
954	dup21q	dup	20,539,750	27,739,250	–	–	–	–	–	–	7,200	2
36599	del(21)(q11-q22)	del	24,214,750	36,601,250	–	–	–	–	–	–	12,387	2
40201	del(21)(q22.11)	del	33,503,750	34,235,250	–	–	–	–	–	–	0,732	2
40518	45,XX,-21[42/46]/46] ring	del	39,380,250	qter	–	–	–	–	–	–	7,565	2
46620	47,XX,+der(21)(pter-q21.1::q21.3-qter).ish der(21) (wep21+,V1I2yRM2029+)	dup, dup	14,393,750	33,094,250	46,719,750	qter	–	–	–	–	18,926	2
SCA	dup21 bk to telomere, bk between 37.389 and 37.58 Mb	dup	14,271,250	29,128,750	37,843,750	qter	–	–	–	–	23,959	2



**Fig. 2** DYRK1A and BDNF protein levels in LCLs from individuals with DS. DYRK1A protein expression (**a**) and BDNF protein levels (**b**) were obtained from 15 healthy individuals (*Controls*), 15 individuals with DS (*DS*), and individuals with partial aneuploidy of human chromosome

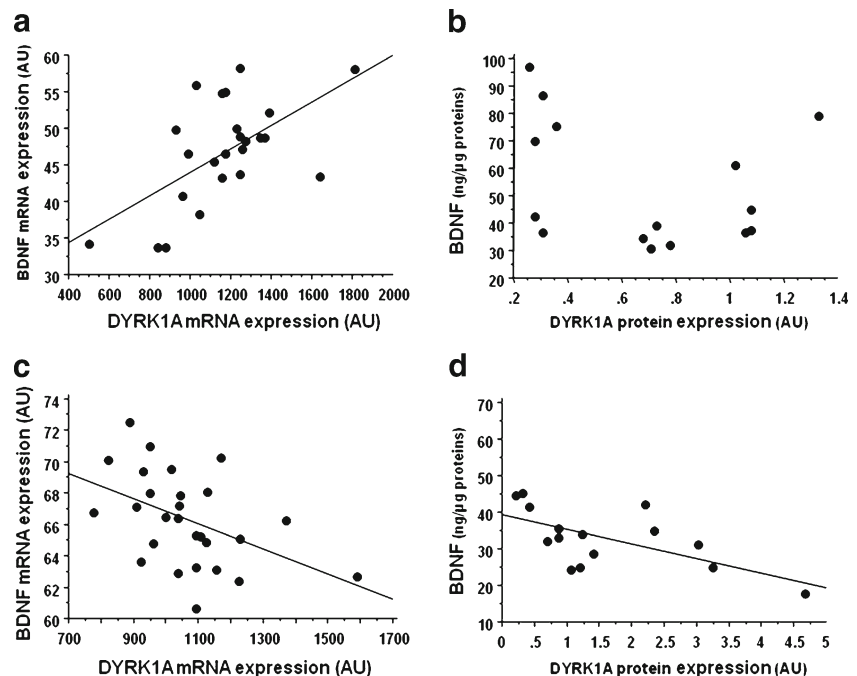
21: six with two *DYRK1A* alleles (*Aneu-2* DYRK1A) and eight with three *DYRK1A* alleles (*Aneu-3* DYRK1A). One-way ANOVA followed by Fisher post hoc test was used for comparison

## Results

BDNF levels were determined in plasma from 24 individuals with DS (age  $50 \pm 10$  years) and 14 healthy controls (age  $50 \pm 10$  years). Plasma BDNF levels were significantly higher in participants with DS than in controls (Fig. 1), consistent with previous findings [14]. To determine the influence of DYRK1A expression on BDNF levels, we analyzed LCLs from individuals with complete aneuploidy for human chromosome 21 (Hsa21) (having three *DYRK1A* alleles; designated as “DS”) and LCLs from individuals with partial aneuploidy for Hsa21 with two (*Aneu-2* DYRK1A) or three (*Aneu-3* DYRK1A) *DYRK1A* alleles (Table 1). These cells were compared to LCLs from control individuals (having only two *DYRK1A* alleles). We first confirmed the overexpression of

DYRK1A in LCLs from individuals with complete trisomy 21 and those with partial trisomy 21 having three *DYRK1A* alleles (Fig. 2a). Expression of DYRK1A was positively correlated with *DYRK1A* copy number ( $r=0.367$ ;  $p<0.0161$ ). Surprisingly, however, in contrast to BDNF expression in plasma from individuals with DS, BDNF expression in LCLs from individuals with DS was significantly decreased (Fig. 2b). A similar nonsignificant decrease in BDNF expression, due to the small number of samples, was found in LCLs from individuals with three *DYRK1A* alleles compared to controls. Conversely, BDNF expression was significantly higher in LCLs from individuals with two *DYRK1A* alleles compared to that in LCLs from individuals with three copies of *DYRK1A* (DS and *Aneu-3* DYRK1A; Fig. 2b). Using human pangenomic Illumina microarrays, our attention was particularly drawn to the BDNF

**Fig. 3** Correlation between BDNF and DYRK1A expression in LCLs. Correlation was assessed with nonparametric Spearman's rank correlation test. Graphs show a positive correlation (including regression line) for mRNA (**a**) ( $r=0.477$ ;  $p<0.0223$ ), no correlation for protein (**b**) expression in healthy individuals, and a negative correlation in individuals with DS for mRNA expression (**c**) ( $r=-0.467$ ;  $p<0.0173$ ) and protein (**d**) ( $r=-0.589$ ;  $p<0.0275$ ) expression



and DYRK1A gene expression. Among the 43 samples from people with DS, 16 were below the threshold significance. We first verified the positive correlation between mRNA *DYRK1A* and DYRK1A protein levels ( $r=0.451$ ;  $p<0.0153$ ). Interestingly, a positive correlation between BDNF and DYRK1A gene expression was found in healthy individuals at the mRNA level (Fig. 3a) ( $r=0.477$ ;  $p<0.0223$ ) without correlation at the protein level (Fig. 3b). However, if we consider that there are two groups of healthy individuals, six controls have very low DYRK1A protein expression (below 0.6 AU) without correlation, but eight controls show a trend toward a positive correlation ( $r=0.688$ ;  $p<0.0518$ ) (Fig. 3b). On the contrary, a negative correlation was found not only at the mRNA level in individuals with DS (Fig. 3C) ( $r=-0.467$ ;  $p<0.0173$ ) but also at the protein levels (Fig. 3d) ( $r=-0.589$ ;  $p<0.0275$ ). Thus, DYRK1A expression affects mRNA and BDNF protein levels in LCLs from DS, but only BDNF mRNA expression in LCLs from healthy individuals.

## Discussion

We have found that the association between trisomy 21 and BDNF levels is opposite for plasma and LCLs when individuals with DS are compared to healthy control individuals. BDNF is stored in human platelets; the concentration in serum is nearly identical to the concentration found in platelet lysates [15]. BDNF is also known to circulate in plasma, and the cellular sources are not clearly defined. We found a large variability in plasma BDNF levels in healthy individuals and those with DS. This could reflect the fact that many cellular sources, like vascular endothelial cells, smooth muscle cells, or activated macrophages or lymphocytes, may contribute to BDNF release in plasma [16–18]. BDNF is also a mediator of angiogenesis [19]. However, no difference was found in spontaneous BDNF release by circulating mononuclear cells [20]. If the endothelium contributes to the variability in BDNF release, it may also have a significant effect on variability of DYRK1A expression.

We demonstrate a negative correlation between BDNF and DYRK1A mRNA expression in LCLs from DS patients, but a positive correlation in LCLs from healthy individuals. Further, our results suggest that at least one locus on Hsa21 may act as a modifier of *DYRK1A* expression and one locus as a modifier of BDNF. BDNF expression is complicated by the existence of multiple promoters [21]. Additionally, CREB and NFAT, transcription factors that are substrates of DYRK1A [22, 23], have been shown to regulate expression of BDNF [24, 25]. There is no evidence to indicate that CREB and NFAT act synergistically or independently on BDNF expression or that they activate transcription from the same promoter [24, 25]. A previous study found that DSCR1 and DYRK1A act synergistically to prevent nuclear occupancy of NFATc

transcription factors, leading to reduced NFATc activity [25]. Thus, reduced NFAT activity may be the link between DYRK1A overexpression and reduced BDNF levels in LCLs from people with DS. The observed variability in BDNF and DYRK1A expression might include a component due to the transformation of the lymphoblastoid cell lines. However, previous studies of murine models of DS [5, 8] and fetal samples [8, 9] suggest that the phenomenon in LCLs mimics that in brains.

The negative correlation found at the mRNA level in LCLs from DS patients was also found at the protein level. Importantly, a recent study demonstrated that Ts65Dn mice, a model of 120 genes in three copies, have lost correlations seen in control mice among levels of functionally related and neurologically important proteins [26]. Loss of normal correlation between BDNF and DYRK1A may underlie deficits in learning and memory. Personality traits are increasingly recognized as endophenotypes in genetic studies of mental disorders. An association has been demonstrated between BDNF polymorphism and anxiety- and depression-related personality traits [27]. In adults with DS, a high proportion of depression and/or anxiety disorders is frequently reported [28]. Given the role of BDNF in neuron maintenance and survival, this finding may explain the deleterious effects of DYRK1A overexpression on learning and memory in people with DS and emphasize the role of genetic variants associated with psychiatric disorders.

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