Scientific Journal of
Neurology & Neurosurgery

Research Article

Protein Expression of ETS2 in Alzheimer's Disease and Down's syndrome as a Personalized Insight: One Gene, One Feature in Common, Diverse-Function-and Diseases -

Mehdipour P[1]*, Javan F[1], Estiar MA[1], Khaleghian M[2], Novin N[1], G Zaeim Kohan H[1], Akhondzadeh S[3]

[1]*Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Department of Medical Genetics, Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Psychiatric Research Center, Roozbeh Psychiatric Hospital, Tehran University of Medical Sciences, Tehran, Iran*

***Address for Correspondence:** Parvin Mehdipour, Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. E-mail: mehdipor@tums.ac.ir

Submitted: 03 December 2016; **Approved:** 06 January 2017; **Published:** 16 January 2017

Citation this article: Mehdipour P, Javan F, Estiar MA, Khaleghian M, Novin N, et al. Protein Expression of ETS2 in Alzheimer's Disease and Down 's syndrome as a Personalized Insight: One Gene, One Feature in Common, Diverse-Function-and Diseases. Scientific Journal of Neurology & Neurosurgery. 2017;3(1): 001-007.

ABSTRACT

Up-regulation of V-ets erythroblastosis virus E26 oncogene homolog 2 (ETS2) may result in increased neuronal vulnerability and degeneration. We aimed to investigate the ETS2 protein expression in the lymphocytes of Down's syndrome (DS) and Alzheimer (Alz) patients who have the neurodegeneration, as the key characteristics in common. Total lymphocyte cells of 69090 and 20130 were analyzed in DS- and Alz.- patients by flow cytometry respectively and confirmed by Immuno-fluorescence. The mean of cells with positive ETS2 was higher in DS than in either the aged-matched control or in Alz patients, it was also higher in females of DS than in Alz patients, but not in in the males of both groups. So, ETS2 has important role in pathogenesis of two different diseases, by reflecting a diverse cellular behavior, with a reliable significant difference for ETS2 protein expression in DS and Alz diseases which harbor two and three chromosome 21, respectively. This fact may pave the way towards the application of more influential strategy by considering the Gene Product-Based Therapy (GDT) and the personalized managements for both diseases as well.

Keywords: Protein Expression; ETS; Down's syndrome; Alzheimer's Disease; Neurodegeneration; Personalized

INTRODUCTION

Trisomy 21 in Down's syndrome is the most prevalent chromosomal disorder that affects 1 in (DS) 850–1,000 infants [1]. Intellectual disability and the early appearance of Alzheimer's disease-like neuropathology are the most prominent features of DS that affect all individuals with this condition [2-5]. Few Down's syndrome cases show the partial trisomy of chromosome 21. This finding proved that only the distal portion trisomy of this chromosome may be responsible for generating the Down's syndrome phenotype and causes profound mental and physical disability [6,7].

Alzheimer's disease, as a degenerative disorder is known by progressive neuronal apoptosis and decline in cognition and distinct neuropathology [8], and the typical rearrangement of *ETS2* gene on chromosome 21 was not found in Alz patients [9]. The identification of the genetic locus causing the familial Alzheimer's disease on chromosome 21q21 [10], revealed not to be located at the same region as in the Down's syndromes, so, this theory would not exclude the involvement of other gene(s) for the sporadic form in other parts of chromosome 21 or rather at another chromosomal locus. Chromosome scattered micro duplications have been stated as an explanation for the increased gene dosage in *ETS2* [11]; and the amyloid protein gene APP [12] in the genome of Alzheimer's disease patients.

V-ets-erythroblastosis virus E26 oncogene homolog 2 (*ETS2*), is located on chromosome 21, and has been correlated with the neurodegenerative phenotype in DS. *ETS2* encodes a transcription factor which promotes the mitochondrial death pathway activation in different DS tissues [13,14]. Down's syndrome primary neuronal cultures demonstrate an approximate five-fold increase in the expression of ETS2 versus control cultures, as opposed to the expected 1.5-fold increase from the gene dosage [14]. Moreover, in the brain of DS patients, high-expression of ETS2 has been correlated with elevated Bax, intracellular β-amyloid and hyper phosphorylated tau, suggesting that up-regulation of *ETS2* may result in increased neuronal degeneration and vulnerability [13]. The aim of this project was to investigate the mode of ETS2 protein expression of lymphocytes in Down's syndrome and Alzheimer's disease patients. Besides, the question is how the same gene with diverse product and a common neurodegenerative feature could lead to two different diseases in patients at different ages?

MATERIALS AND METHODS

Subjects

Ten patients affected with Alzheimer disease (minimum age: 42 and maximum: 79 years); and 10 with Down's syndrome (minimum age:1; maximum:14) after given an informed written consent and approval of the ethics committee of Tehran University of Medical Sciences participate in this study (Tables 1,2). For each group of patients, 10 separate normal individuals as the age-matched controls were included in this investigation; minimum and maximum age of controls were 2-13 years for Donwn's control and 42-81 years for the Alzheimer's control (Tables 3, 4). The whole peripheral blood samples were collected in standard 5-ml sodium heparin tubes.

Flow cytometry

For cell extraction, lymphocytes from whole blood were cultured in RPMI-1640 media (Sigmae Aldrich, St Louis, MO, USA) for 30 min at 37°C. Cells were washed twice P.B.S. and fixed in Methanol (Merck, Germany).

The lymphocytes were stained with polyclonal ETS2 antibody, isotype rabbit Ig (Aviva Systems Biology, CA, USA). Cells were washed twice by PBS, then, FITC-conjugated goat-anti-rabbit secondary antibody was used. The untreated cells were tested as negative control for the same cell population.

Flow cytometry assay was performed by BD FACS Calibur flow cytometry (BD FACSCALIBUR™ Flow Cytometry System, US) and the results were analyzed using flowjo software (Flowjo 7.6).

Immunofluorescence assay

For cell extraction, lymphocytes from whole blood were cultured. Then cells were fixed in Methanol twice.

Staining of cells was performed by polyclonal anti-ETS2 with the FITC conjugated goat-anti-rabbit secondary antibody.

Statistical analyses

Data was statistically analyzed using SPSS 18 (SPSS Inc, IL, USA) and Graph Pad Prism 6 (GraphPad Prism Software Inc, San Diego, CA) software's. Shapiro-Wilk, Mann-Whitney U, Kruskal-Wallis, Fisher Exact test, and Chi-Square tests. The Spearman and Pearson Correlation Coefficients were also performed. In all of the statistical analysis, p value less than 0.05 was considered as significant.

RESULTS

Total cells of 69090 and 20130 were analysed by flow cytometry in patients affected with Alz disease and DS respectively (Tables 1, 2). The flow cytometry result of ETS2 protein expression in lymphocytes of an Alzheimer disease patient is illustrated (Figure 1).

The ETS2 protein expression of lymphocyte by Immuno

Table 1: ETS2 protein expressions status and clinical features of Alzheimer's disease patients.

No	Gender	Clinical features	Family history of Alz	Age (Y)	ETS2 protein expression (%)	Total cells evaluated by flow cytometry
1	Female	ML		65	146 (3.47)	4207
2	Female	ML, Tremble (hands)	CPF	56	155 (2.13)	7276
3	Female	ML,MD		78	121 (1.11)	10900
4	Male	ML,	HB	74	607 (15.8)	3841
5	Male	ML,		79	810 (11)	7363
6	Male	ML(severe)		62	166 (2.43)	6831
7	Male	ML(severe)	-	42	1100 (6.75)	16296
8	Female	ML		54	228 (3)	7600
9	Female	ML,HC,BP	MA	45	98 (5.12)	1914
10	Female	ML		79	541 (18.9)	2862

Total cells analyzed in 10 patients by flow cytometry: 69090
ML: memorial loss, MD: movement disorder
HC: heart complication, BP: high blood pressure
Alz: Alzheimer disease; (Y): age of onset in years
CPF: Cousin of paternal Father
HB: Half Brother
MA: Maternal Aunt

Table 2: ETS2 protein expressions status and clinical features of Down's syndrome patients.

No	Gender	Clinical features	Age (Y)	Family history of DS	ETS2 protein Expression (%)	Total cells evaluated by flow cytometry
1	Male	HC	3	-	205 (6.02)	3402
2	Male		2		85 (6.34)	1340
3	Female		5	-	501 (5.48)	9133
4	Female	HC	3		32 (24.8)	129
5	Male		7	-	99 (7.04)	1405
6	Male		6	-	34 (15.11)	225
7	Male		1	-	102 (17.08)	597
8	Male		3	-	100 (18.21)	549
9	Female		12	-	35 (26.71)	131
10	Female		14	-	201 (6.24)	3219

Total cells analysed in 10 patients: 20130
DS: Down's syndrome
HC: Heart Complication

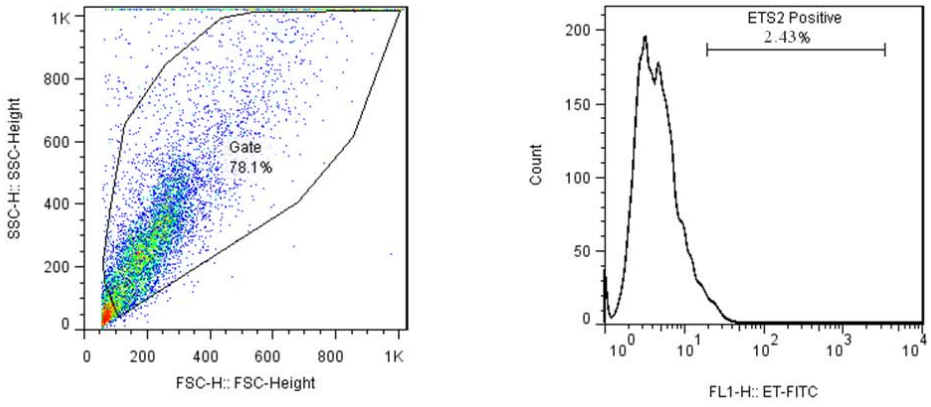

Figure 1: The flow cytometry results of ETS2 protein expression in lymphocytes from a patient affected with Alzheimer disease
R1: 7812/10000 (78.1 %)
ETS2: 2.43 % (190/7812)

fluorescence is provided in a patient affected with DS (Figure 2), in Alz (Figure 3), and in control (Figure 4).

Regarding the sample size, although the numbers of patients were limited either for AL or DS, but the number of analyzed cells were considered as the target population in this study (Tables 3,4).

The mean of cells with positive protein expression is found to be 139.4 ± 44.71 (min: 32, max: 501) for DS and for the aged-matched control was 3.2 ± 0.61(min: 1, max: 6). These figures revealed to

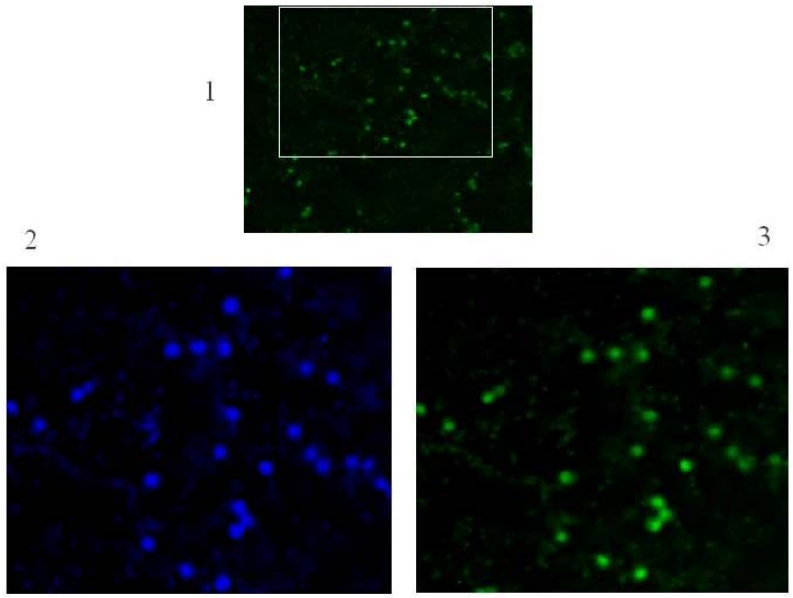

Figure 2: Protein expression of ETS2 in a patient affected with Down's syndrome
Lymphocytes with dapi (magnification x100)
Lymphocytes with dapi (magnification x200)
Same lymphocytes with FITC, reflective of low expression of polyclonal antibody ETS2 (magnification: x200)

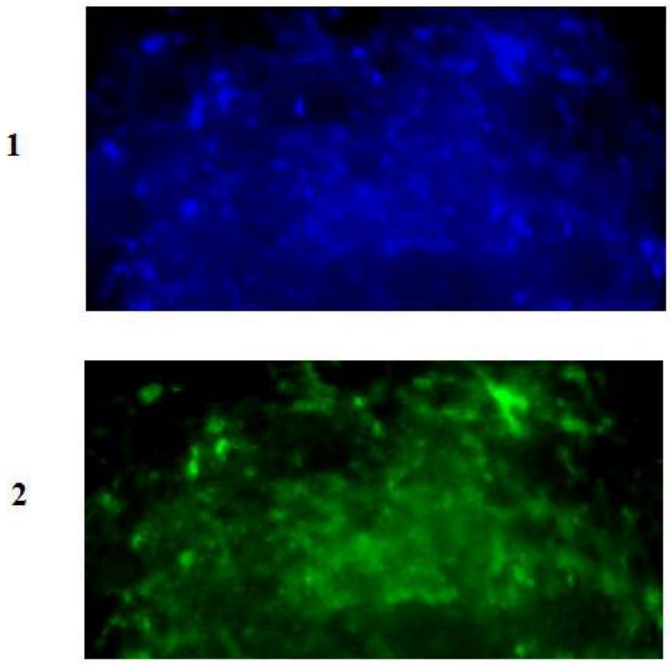

Figure 3: Protein expression of ETS2 in a patient affected with Alzheimer disease
Lymphocytes with dapi
Same lymphocytes with FITC, reflective of the diverse protein expression with polyclonal antibody ETS2

be mean: 397.2 ± 1.11 (min: 98 max: 1100) for Alzheimer's disease patients, and mean: 3.2 ± 0.61 (min: 1, max: 6) for controls. Besides, data was normally distributed. There was statistically significant difference between patients and aged-matched control for DS (P = 0.007, t = 3.046, df = 18) and 95% confidence interval was 42.25 to 230.14. The Statistics Ratio for control / Down's syndrome is revealed to be 164.5 %.There was also statistically significant difference between patients and control for Alz (P = 0.006, t = 3.56, df = 9.001) and 95% confidence interval was 143.45 to 643.34.

The Statistics ratio for control / Alzheimer was 264.7 %. Furthermore, there was statistically significant difference between the patients affected with Alz and DS (P = 0.044, t = 2.16, df = 18) and 95% confidence interval was 7.38 to 508.21. Ratio Statistics for Alzheimer's

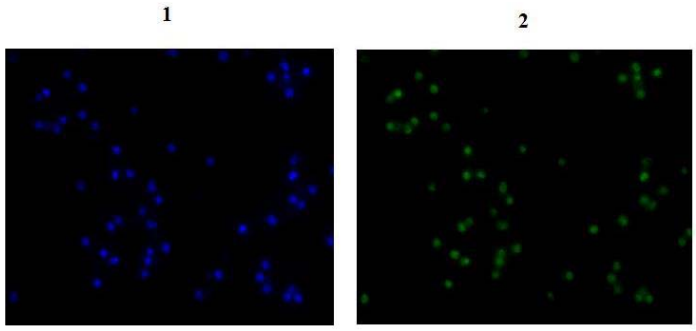

Figure 4: Protein expression of ETS2 in a healthy individual as control
Lymphocytes with dapi (magnification: x100)
Same lymphocytes with FITC. Lack of expression is observable in majority of cells with polyclonal antibody ETS2(magnification: x100)

Table 3: ETS2 protein expressions status in healthy individuals as control for patients with Alzheimer disease.

ID	Age(Y)	ETS2 protein expression (%)	Total cells analysed
C-1	65	2 (0.066%)	3025
C -2	56	10 (0.19%)	5214
C -3	79	0 (0%)	1546
C -4	74	3 (0.065%)	4587
C 5-	79	6 (0.48%)	1232
C -6	62	3 (0.38%)	783
C -7	42	0 (0%)	923
C -8	54	4 (0.10%)	9823
C -9	81	7 (004%)	874
C -10	79	3 (0.19%	1542
Total		**38 (0.12%)**	**29549**

The cells were analysed by flow cytometry:
All controls (C) had no family history of Alzheimer disease

disease / Down's syndrome is found to be 234.6%. Expression of ETS2 protein was significantly higher in Down's syndrome than in Alzheimer's disease patients.

The ETS2 protein expression was significantly higher in females of Down's syndrome - than in Alzheimer's disease patients (P = 0.013). However, no significant difference of ETS2 protein expression was found in the male patients of both groups (Figure 5).

DISCUSSION

Apoptosis plays the crucial role in neuro degeneration and the Alzheimer's disease-neuropathology in Down's syndrome. Both effects seem to be due to the high-expression of genes that either directly modify the cell death pathways or indirectly modify them through the alterations in the genes' expression that enhance oxidative stress or induce Alzheimer's disease-like pathology, i.e. APP, rendering the cell more vulnerable to apoptosis-inducing factors [16]. In addition, Wolvetang, et al. [14] proposed that the ETS2 over expression is the key contributor to the high susceptibility of Down's syndrome cells to apoptotic stimuli.

Molecular study, i.e., "quantitative densitometry" revealed that the extra copy of *ETS2* gene was the cause of phenotypic feature of the patients affected with DS [11]. However, as far as the Alzheimer disease concerns, the region of *ETS2*, is found to be different in Alzheimer disease than in normal individuals by In Situ Hybridization technique [17], and was in concordant with the proposed hypothesis that trisomy of one/more gene in the Down's syndrome region might be the cause of Alzheimer's disease as well [18]. However, these reports are rather challenging due to genotype / phenotype discordance. However, in our study, there is no concern regarding the region of *ETS2* gene, but the product of gene, i.e., the statue of ETS2- protein expression is the matter of fact. Our findings also reflect the heterogeneity of expression which may be as a result of *mosaicism* which could not be detected by the molecular techniques, so, in addition to the performance of protein expression assay, it is essential to classify the mode of chromosomal aberration in patients with Down's syndrome. It's also worth to state that the Down's syndrome patients with Robertsonian translocation, t(D;G), could also be classified in those group with involvement of other chromosomal region, rather than chromosome 21 as a sole. So, the protein- based assay of *ETS2* gene could be considered as a core, reliable and a complementary strategy for Alz and DS patients.

Also, it was also stated by other authors that ETS2 is highly expressed in Down's syndrome [19-21]. High expression of ETS2 induces neuronal apoptosis, suggesting that ETS2 with high-expression may participate to an increased rate of apoptosis of neurons in Down's syndrome [22]. ETS2 protein trans-activates APP gene and fibroblasts high-expressing ETS2 display molecular abnormalities seen in Down's syndrome, such as increased expression of APP gene and elevated β-amyloid proteins [14], suggesting that ETS2 high-expression in DS determines high-expression of APP

Table 4: ETS2 protein expressions status in healthy individuals as control for patients with Down's syndrome.

ID	Age (Y)	ETS2 protein expression (%)	Total cells analysed
C-1	4	1 (0.066%)	2108
C -2	2	3 (0.19%)	3876
C -3	3	2 (0%)	942
C -4	5	4 (0.065%)	2361
C 5-	8	1(0.066%)	765
C -6	13	4 (0.38%)	435
C -7	10	1 (0.066%)	1368
C -8	9	6 (0.08%)	6734
C -9	7	4 (004%)	657
C -10	10	6 (0.19%)	3489
Total		32 (0.14%)	22735

The cells were analysed by flow cytometry:
All controls (C) had no family history of Down's syndrome

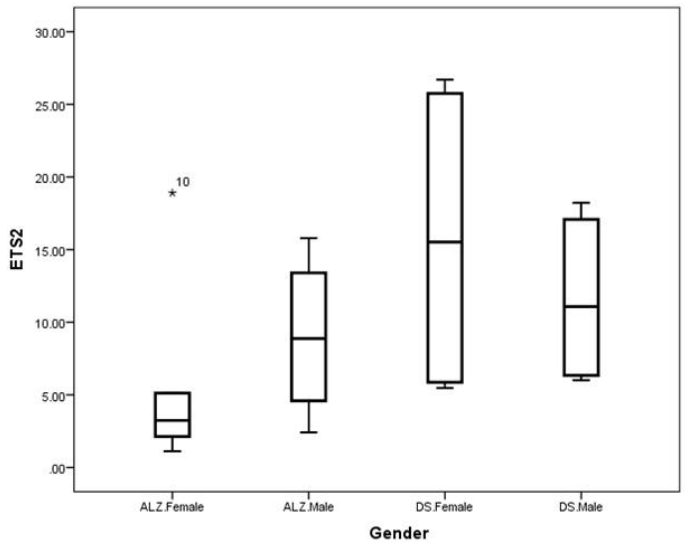

Figure 5: The comparison of ETS2 protein expression in Down syndrome and Alzheimer disease of male and female groups.

and may play a role in the pathogenesis of the brain abnormalities in Alz and DS. However, DS patients had high level of ETS2 protein expression in comparison to Alz patients. Particularly, the diverse level of this protein was significant for female patients of both groups.

Due to the key roles of *ETS2* gene in regulating cellular growth and differentiation as well as development of organism, it is essential to explore the nature of functional alteration such as protein expression which is considered as the final destination of any gene alteration, and most importantly the occurrence of mutation. In spite of remarkable Scientific and clinical progression, a lot of questions remain without or with partial responses, therefore the supplementary efforts are required in different domains to decipher how the gene over dosage generate the mental retardation in Down's syndrome. Regarding the importance of diversity in gene expression and pathogenesis in Down's syndrome, it is clear that protein expression assay is essential to become as a routine tool to detect more classified pattern of the ETS2 protein expression and as a reliable functional assay in Down's syndrome. In addition, performance of protein expression is more Informative and comprehensive than the analysis of the molecular assay as a sole. Therefore, the functional characterization of the encoded proteins by the genes located on HC21, as they are involved in the brain development and cognition are required to be unmasked. Overall, proteome studies will help to understand the final effect of the protein as the *ETS2* gene product in blood of Down's syndrome patients.

It could be concluded that the ETS2 protein expression has important role in pathogenesis of two different diseases which have a neurodegenerative disorder in common. Our data reflects a diverse cellular behavior with a reliable significant difference for ETS2 protein expression in Alzheimer disease and Down's syndrome. Interestingly, all of our teen ager patients with typical trisomy 21 reflect higher protein expression than the patients affected with Alzheimer disease within the range of 42 to 79 years old. In this regard, the problems do not rely on the age of onset. However, the *hypothetical fact is the gene/production* which has an influential capability to create a remarkable neurodegenerative status in the patients with Down's syndrome and Alzheimer disease. Furthermore, the other concerns and questions on the manner of *ETS2* gene function include the following items:

1. How does it act within the early onset Alzheimer patients?
2. How does it act within the Down's syndrome patients with the Robertsonian translocations including t (D;G)?
3. How does it act within the mosaics' DS?
4. How is the correlation between mode of ETS2 protein expression and severity of neurodegeneration in Down's syndrome and Alzheimer disease?

As final words, the ETS2gene is a fascinating gene, with diverse function in two different diseases.

REFERENCES

1. Shin M, Besser LM, Kucik JE, Lu C, Siffel C, Correa A, et al. Prevalence of Down syndrome among children and adolescents in 10 regions of the United States. Pediatrics. 2009; 124(6): 1565-1571.
2. Sabbagh MN, Fleisher A, Chen K, Rogers J, Berk C, Reiman E, et al. Positron emission tomography and neuropathologic estimates of fibrillar amyloid-β in a patient with Down syndrome and Alzheimer disease. Archives of neurology. 2011; 68(11): 1461-1466.
3. Cenini G, Dowling AL, Beckett TL, Barone E, Mancuso C, Murphy MP, et al. Association between frontal cortex oxidative damage and beta-amyloid as a function of age in Down syndrome. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2012; 1822(2): 130-138.
4. Nadel L. Down's syndrome: a genetic disorder in biobehavioral perspective. Genes, Brain and Behavior. 2003; 2(3): 156-166.
5. Vicari S. Memory development and intellectual disabilities. Acta Paediatrica 2004; 93(445): 60-63.
6. Huret JL, Delabar JM, Marlhens F, Aurias A, Nicole A, Berthier M, et al. Down syndrome with duplication of a region of chromosome 21 containing the CuZn superoxide dismutase gene without detectable karyotypic abnormality. Human genetics 1987; 75(3): 251-257.
7. Delabar JM, Sinet PM, Chadefaux B, Nicole A, Gegonne A, Stehelin D, et al. Submicroscopic duplication of chromosome 21 and trisomy 21 phenotype (Down syndrome). Human genetics. 1987; 76(3): 225-229.
8. Arendt T, Brückner MK, Morawski M, Jäger C, Gertz HJ. Early neurone loss in Alzheimer's disease: cortical or subcortical? Acta neuropathologica communications. 2015; 3: 1-11.
9. Sacchi N, Nalbantoglu J, Sergovich FR, Papas TS. Human ETS2 gene on chromosome 21 is not rearranged in Alzheimer disease. Proceedings of the National Academy of Sciences. 1988; 85(20): 7675-7679.

10. St George-Hyslop PH, Tanzi RE, Polinsky RJ, Haines JL, Nee L, Watkins PC, et al. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. Science. 1987; 235(4791): 885-890.

11. Delabar JM, Lamour Y, Gegonne A, Davous P, Roudier M, Nicole A, et al. Rearrangement of chromosome 21 in Alzheimer's disease; 1985; 29(4): 226-228.

12. Delabar JM, Goldgaber D, Lamour Y, Nicole A, Huret JL, de Grouchy J, et al. Beta amyloid gene duplication in Alzheimer's disease and karyotypically normal Down syndrome. Science. 1987; 235(4794): 1390-1392.

13. Helguera P, Pelsman A, Pigino G, Wolvetang E, Head E, Busciglio J, et al. ets-2 promotes the activation of a mitochondrial death pathway in Down's syndrome neurons. The Journal of neuroscience. 2005; 25(9): 2295-2303.

14. Wolvetang EJ, Wilson TJ, Sanij E, Busciglio J, Hatzistavrou T, Seth A, et al. ETS2 over expression in transgenic models and in Down syndrome predisposes to apoptosis via the p53 pathway. Human molecular genetics. 2003; 12(3): 247-255.

15. Sanij E, Hatzistavrou T, Hertzog P, Kola I, Wolvetang EJ. Ets-2 is induced by oxidative stress and sensitizes cells to H 2 O 2-induced apoptosis: implications for Down's syndrome. Biochemical and biophysical research communications. 2001; 287(4): 1003-1008.

16. Rueda N, Flórez J, Martínez-Cué C. Apoptosis in Down's syndrome: lessons from studies of human and mouse models. Apoptosis 2013; 18(2): 121-134.

17. Blanquet V, Turleau C, Stehelin D, Creau-Goldberg N, Delabar J, et al. Regional mapping of ETS2 on chromosome 21 is different in a normal individual and in an Alzheimer disease patient. Human Gene Mapping 9, 9, Paris, 1987, Cytogenet cell Genet, 46(1-4): 583.

18. Schweber M. A possible unitary genetic hypothesis for Alzheimer's disease and Down syndromea. Annals of the New York Academy of Sciences. 1985; 450: 223-238.

19. Dauphinot L, Lyle R, Rivals I, Dang MT, Moldrich RX, Golfier G, et al. The cerebellar transcriptome during postnatal development of the Ts1Cje mouse, a segmental trisomy model for Down syndrome. Human molecular genetics. 2005; 14(3): 373-384.

20. Lyle R, Gehrig C, Neergaard-Henrichsen C, Deutsch S, Antonarakis SE. Gene expression from the aneuploid chromosome in a trisomy mouse model of down syndrome. Genome research. 2004; 14(7): 1268-1274.

21. Saran NG, Pletcher MT, Natale JE, Cheng Y, Reeves RH. Global disruption of the cerebellar transcriptome in a Down syndrome mouse model. Human molecular genetics. 2003; 12(16): 2013-2019.

22. Wolvetang EJ, Bradfield OM, Hatzistavrou T, Crack PJ, Busciglio J, Kola I, et al. Over expression of the chromosome 21 transcription factor Ets2 induces neuronal apoptosis. Neurobiology of disease. 2003; 14(3): 349-356.