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EARLY DIAGNOSIS AND INTERVENTION OF DEVELOPMENTAL DYSLEXIA AT THE PRESCHOOL AGE: THE ROLE OF STRESS

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Abstract

Developmental dyslexia (DD) is a multifactorial, specific learning disorder characterized by various interactions of dysfunctions of biological, neurophysiological, cognitive, and psychomotor factors. In this study the association between the early signs of DD and stress was investigated. Polymorphisms/variants that affect the expression levels of HPA axis related genes - that are involved in the regulation of stress response- were studied along with the mitochondrial DNA copy number (mtDNAcn), a sensitive stress biomarker. 314 children aged 5.0 to 6.0 years were recruited, while 20 preschoolers were identified 'at risk' of dyslexia, along with 10 typically developed preschoolers (control group). From the 'at risk' of DD group 10 individuals underwent a 3-month systematic intervention program. Two screening tests for early identification of DD were administered, while a developmental history and the CBCL 1½–5 form of the Achenbach System of Empirically Based Assessment (ASEBA) were completed. Buccal DNA was extracted. Genotyping of variants of HPA axis genes was performed along with mtDNAcn estimation before and after the intervention in all participants.

Multivariate analysis was applied between all variables between the three groups. A statistically significant difference was observed between the DD and typically developed group on cognitive, psychomotor, and linguistic factors, along with similar statistically significant difference recorded within the DD group before and after the intervention. Although no statistically significant difference was observed before the intervention in mtDNAcn, after the intervention, a statistically significant difference was observed in the intervention group compared to the other 2 groups. Early dysfunctions in specific factors are revealed in a complex framework of interactions, shaping an early DD phenotype. Stress is considered to play an important role in the early occurrence of DD. Early detection of DD in preschool becomes more urgent as it contributes to the implementation of effective interventions, thereby reducing or preventing multiple negative effects in later school age.

Keywords: *Developmental Dyslexia, early identification, early intervention, multifactorial phenotype, stress.*

1. Introduction

Developmental dyslexia (DD) is a multifactorial, specific learning disorder characterized by multiple dysfunctions of one or more, biological, neurophysiological, psychomotor, cognitive, and socioemotional factors (Livingston et al., 2018). The onset of symptoms of DD, while systematically recorded and addressed during the school age, becomes apparent from the preschool age onwards (Zakopoulou et al., 2023). Several independent studies support that frustration, failure and difficulties caused by learning difficulties, create a constant fear of failure or real failure, sadness, inadequacy, reduced happiness and self-esteem, stress, anxiety, emotional vulnerability (Exarchou et al., 2020; Peterson, 2021; Zakopoulou et al., 2019). All these matters can influence an individual's predisposition to DD, regardless of the presence or absence of genetic variations in risk genes (Romeo et al., 2018). In the context of DD etiopathogenesis, stress is considered as an important factor (Theodoridou, et al., 2021), presumably underlying a dysregulation of the HPA axis (Kershner, 2020). *FKBP5*, the overexpression of SNV rs1360780 inside intron 2 of the gene, the levels of *SLC6A4*, 5-HTTLPR polymorphic region,

rs25531 SNV inside the gene's promoter region, and the mitochondrial DNA copy number (mtDNAcn), a sensitive stress biomarker, influence the response to stress (Palma-Gudiel & Fananas, 2017).

However, regarding the study of the existence of an early interactive relationship between stress and DD, as well as the importance of this interaction in a multifaceted understanding of the endophenotype and phenotype of DD, a critical literature gap is highlighted.

2. Objectives

Aiming to a comprehensive understanding of this phenotype, the purpose of this study is to investigate the early role of stress in this network of strongly interrelated difficulties, through an innovative research protocol.

3. Methods

In this paper we present the results of the analysis investigating the association between the early signs of DD and the HPA axis genes involved in the regulation of stress response and the polymorphisms/variants affecting their expression levels, while mitochondrial DNA copy number (mtDNA cn), a sensitive stress biomarker, was also evaluated.

3.1. Data acquisition

307 children aged 5.0 to 6.0 years participated in this study, consisting of three testing groups, as follows: 10 preschoolers were identified 'at risk of DD with intervention' (they underwent a 3-month systematic intervention program), 10 'at risk of DD without intervention' (they underwent no intervention program), and 10 typically developed preschoolers, 'without DD'. In no one of the subjects was recorded co-occurrence with other neurodevelopmental disorders. Written consent forms were obtained from all parents of the children who participated on a voluntary basis.

3.1.1. Material. Towards early identification of DD, the test of Early Dyslexia Identification (EDIT) (Zakopoulou, 2003) and the ATHINA test (Paraskevopoulos et al., 1999) were administered. Seeking to compare any early signs of DD with early internalizing and externalizing problems (Emotionally Reactive, Anxious, Depressed, Aggressive Behavior, Attention Problems, Somatic Complaints, and Withdrawn), a developmental history and the Greek version of the Child Behavior Checklist for Ages 1½ to 5 (CBCL 1½–5) of the Achenbach System of Empirically Based Assessment (ASEBA) (Roussou, 2009) were completed.

Through the EDIT test three sectors (including 8 tasks) were examined, considering: (i) Visual-spatial Abilities (Sketching, Copying shapes, Visual discrimination/Laterality/Left-right discrimination), (ii) Grapho-phonological Awareness (Phonemes Composition; Phonemes Discrimination; Name Writing) and (iii) Working Memory (Phonemes Discrimination, Name Writing, Copying shapes, Visual-verbal correspondence). Through the ATHINA test one sector (Short-term Sequence Memory (Numbers Memory, Pictures Memory, Shapes Memory) was examined. To examine the role of the stress at the molecular level, DNA from buccal cell swabs was extracted from all subjects: (a) mtDNAcn was evaluated by qPCR, using primers to target the nuclear DNA and mtDNA. One sample from the "at risk of DD with intervention" group was considered an outlier and removed from the analysis; (b) rs1360780 was genotyped using a TaqMan assay (Thermo Assay ID: C_8852038_10, #4351379, Applied Biosystems, Foster City, CA), while 5-HTTLRP and rs25531 were analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

3.2. Statistics

Multivariate analysis was applied between all variables between the three groups. Data were examined for normality distributions, and non-parametric tests were used in cases where data did not follow Gaussian distribution.

4. Results

A statistically significant difference was observed between the DD and typically developed group on cognitive, psychomotor, and linguistic factors, along with similar statistically significant difference recorded within the DD group before and after the intervention (see table 1).

Table 1. Differences between the groups of children at risk of DD with intervention and children without early signs of DD.

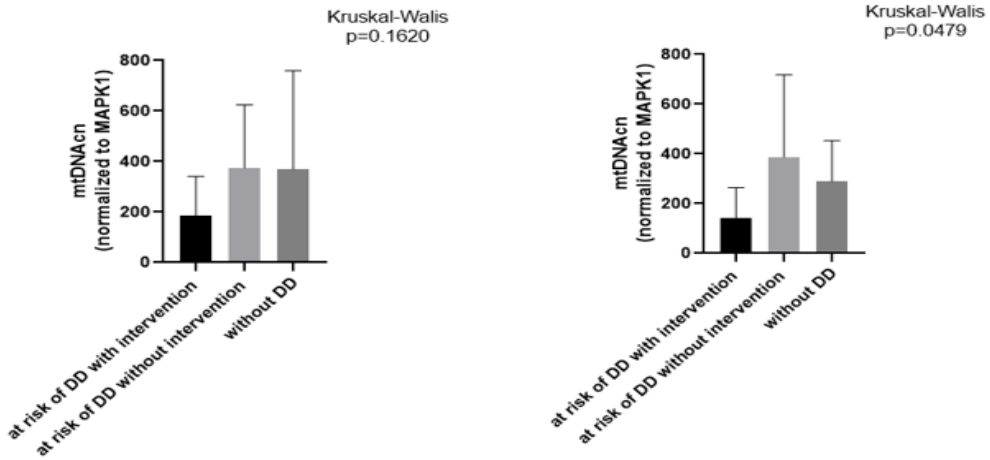
Tested Skills	Groups									
	At risk of DD with intervention					Without DD				
	Mean	SD	Median	Range	P	Mean	SD	Median	Range	P
Sk_ initial	2.90	2.51	2.50	6.00	0,007	8.90	3.41	9.50	12.00	1,000
Sk_ final	6.80	2.70	8.00	7.00		8.90	3.41	9.50	12.00	
Csh_ initial	2.80	2.44	2.00	8.00	0,012	7.50	.97	8.00	3.00	1,000
Csh_ final	6.30	1.34	6.00	4.00		7.50	.97	8.00	3.00	
VD_ initial	5.70	.48	6.00	1.00	0,317	5.90	.32	6.00	1.00	1,000
VD_ final	5.90	.32	6.00	1.00		5.90	.32	6.00	1.00	
L_ initial	5.20	2.57	6.00	8.00	0,227	5.90	2.96	6.00	10.00	1,000
L_ final	6.00	2.91	5.50	8.00		5.90	2.96	6.00	10.00	
L-R_ initial	2.50	1.18	2.50	4.00	0,238	2.00	.82	2.00	3.00	1,000
L-R_ final	2.50	1.18	2.50	4.00		2.00	.82	2.00	3.00	
NW_ initial	2.80	1.93	3.50	5.00	0,007	7.70	.48	8.00	1.00	1,000
NW_ final	5.90	1.85	6.00	5.00		7.70	.48	8.00	1.00	
PhD_ initial	6.50	.71	7.00	2.00	0,005	9.50	.71	10.00	2.00	1,000
PhD_ final	8.90	.74	9.00	2.00		9.50	.71	10.00	2.00	
V-VC_ initial	1.60	2.32	.00	6.00	0,068	.00	.00	.00	.00	1,000
V-VC_ final	.00	.00	.00	.00		.00	.00	.00	.00	

Note. Sketching= Sk; Copying shapes=Csh; Visual discrimination=VD; Laterality=L; Left-right discrimination=L-R; Name Writing=NW; Phonemes Discrimination=PhD; Visual-Verbal correspondence=V-VC

From the Buccal DNA analysis, the following results were recorded:

I. mtDNA copy number: although no statistically significant difference was observed before the intervention in mtDNAcn between the three groups, after the intervention, statistically significantly lower mtDNAcn levels were recorded in the group *at risk of DD with intervention*', compared to the other 2 groups, (Kruskal Wallis, p=0.048) (see figure 1). Specifically, as we see in the table, the measurements are based on groups but also the time. The changes that were observed across time are in all cases non-significant even though the central tendency expressed by the median is indicative of differences. The main statistically significant difference recorded was between the '*at risk of DD with intervention*' group and the 2 other groups, where significantly smaller values of stress were detected' (p=0.048).

Figure 1. mtDNA copy number differences between the three groups at the initial and final testing state (after three months)



I. Genotyping of variants of stress related genes: Although the “risk” alleles are more common in the dyslexia group, no statistically significant difference is observed (see table 2).

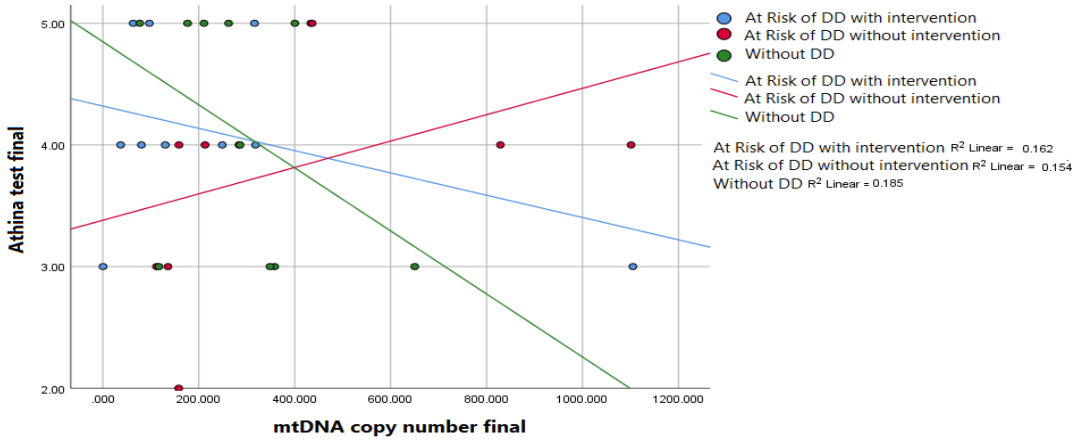
Table 2. Allelic frequencies of genotyped variants (risk alleles are in bold).

	At risk of DD (n=20)	Without DD (n=10)	*p*	At risk of DD (n=40)	Without DD (n=20)	*p*
SLC6A4 5-HTTLRP	SS (5/20 - 25%) SL (9/20 - 45%) LL (6/20 - 30%)	SS (1/10 - 10%) SL (5/10 - 50%) LL (4/10 – 40%)	*0,775*	S (19/40-47,5%) L (21/40-52,5%)	S (7/20-35%) L (13/20-65%)	*0,519*
FKBP5 rs1360780	CC (8/20 - 40%) CT (11/20 - 55%) TT (1/20 - 5%)	CC (6/10 - 60%) CT (4/10 - 40%) TT (-)	*0,633*	C (27/40-67,5%) T (13/40-32,5%)	C (16/20-80%) T (4/20-20%)	*0,478*
SLC6A4 rs25531	AA (17/20 - 85%) AG (3/20 – 15%) GG (-)	AA (9/10 - 90%) AG (1/10 - 10%) GG (-)	*1*	A (37/40 - 92,5%) G (3/40 – 7,5%)	A (19/20 - 95%) G (1/20 – 5%)	*1*

II. Furthermore, the effect of several indices was examined on these differences. Specifically, the observed mtDNA measurements were examined for differences depending on the presence of internalizing and externalizing problems, according to the answers on CBCL 1½–5. None of these had a statistically significant effect on mtDNA values, before or after the intervention or to their change.

III. A series of correlations were also examined between scores at the EDIT and ATHINA test with the mtDNAcn values and the mtDNAcn observed changes. A statistically significant and positive correlation was observed for the group ‘at risk of DD without intervention’ highlighting a positive correlation between the persistence of the difficulties and an increase in stress. Regarding the mtDNA changes not statistically significant and correlations were observed for any group.

Figure 2. Correlations between ATHINA scores and mtDNAcn values at the final testing stage.



4. Discussion

Children ‘at risk’ of DD meet difficulties in a wide range of skills in a complex framework of interactions, shaping an early DD phenotype. The main aim of the current study was to investigate the role of the stress in the early DD phenotype. Based on the results of this study, we see that the children who were diagnosed at risk of DD at the initial testing stage, showed a statistically significant improvement in all the domains that initially had recorded low scores, after the implementation of the intervention. The findings underscored that the stress-related risk alleles are more common in children at risk of DD, while mtDNAcn displayed lower levels in the “at risk of DD with intervention” group and did not show statistically significant differences with typically developing children before the intervention. Despite these low levels of mtDNAcn, a reduction in mtDNAcn was observed after the intervention, indicating that early intervention programs contribute positively to minimizing stress levels, confirming relevant research findings. Adding to this, the significant correlation observed between stress and the persistence of difficulties in specific domains, such as working memory, rather confirms the Horbach’s et al., (2019) statement that internalizing problems increase numerically during the transition from

kindergarten to elementary school. In the light of these findings, it is highly suggested that DD phenotype is a stress-related phenotype that could help to alleviate negative psychological conditions, such as stress, and to prevent the development of behavioral problems.

5. Conclusions

A stress-related DD phenotype indicate the existence of powerful mechanisms that negatively influence the reduction or prevention of multiple later school age difficulties and personality effects in individuals with DD.

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