RESEARCH PAPER

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Assessment of the relationship between Val66Met BDNF polymorphism and the effectiveness of gait rehabilitation in children and adolescents with cerebral palsy

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Cerebral palsy (CP) is associated with the non-progressive damage of upper motor neurons, which is manifested by a variety of symptoms, particularly motor and functional deficits. During the rehabilitation of patients with CP, attention is paid to improving mobility which can have a significant impact on the child's development. The effectiveness of rehabilitation depends on the plasticity of the nervous system, which may be genetically determined. Of importance are the various polymorphisms of the brain derived neurotrophic factor (*BDNF*) gene. It has been shown that the Val/Val genotype may predispose children to greater improvements in function and its maintenance. However, subjects with the Met allele showed a reduced tendency to improve their motor functions but had significantly better results on indirect tests assessing gait function. Fifty subjects with CP participated in this study. They were divided into two groups by genotype and examined on their rehabilitation progress in terms of improved gait function. The results correlated with other studies describing the relationship between the *BDNF* genotype and learning motor functions in CP, and with numerous studies on the relationship between *BDNF* genotype and neuroplasticity in stroke patients. This research provides a basis for the identification of genetic biomarkers in patients with CP which can be used to predict the effects of rehabilitation therapy and help with the development of personalized treatments.

Key words: BDNF, cerebral palsy, motor learning, neuroplasticity, rehabilitation robotics

INTRODUCTION

Cerebral palsy (CP) is the most common cause of disability among children and adolescents. CP is defined as a set of symptoms arising from the non-progressive damage of the central nervous system. This damage can change with age, cause movement and posture disorders, and result in other consequences of brain damage. Limitations in motor functions are often accompanied by other disorders, e.g., cognitive, communication, mental, and sensory disorders. Due to the inability to reverse the root cause of damage to the upper motor neurons, medical treatments should focus on minimizing the consequences of the damage by improving motor and cognitive functions, stimulating motor activity, and enabling participation in social life. In this regard, the biggest obstacle for patients with CP

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and their parents/caregivers is the impaired development of gait functions, which limits the opportunities to participate in social activities.

Approximately 70% of children with CP have a chance to walk with over 50% of them being able to accomplish this independently. Improving a patient's mobility can have a positive effect on their development, therefore special attention should be paid to improving locomotion (Beckung et al., 2008; Maenner et al., 2016). Technological advancements and new physiotherapy methods focused on functional assessment and technology-assisted rehabilitation to improve the quality of rehabilitation in children with CP. Using a three-dimensional, instrumental assessment of gait kinematics provides an objective assessment allowing for the identification of incorrect components within the complex problem of locomotion disturbances. In neurorehabilitation, the plasticity of the nervous system is of particular importance (Stavsky et al., 2017).

Neuroplasticity is the adaptive feature of the nervous system responsible for changes within the system at the structural and functional levels. It is necessary for processes such as variability, learning, and self-repair to occur (Voss et al., 2017). As well as, being the basis for the proper development of the nervous system and neurologic rehabilitation (Kolb and Gibb, 2011; Keci et al., 2019). The plasticity of the nervous system occurs due to the processes such as synaptogenesis, neurogenesis, and changes in the strength of interneuronal connections (Kania et al., 2017). During the processes of memory and learning, plasticity corresponds, inter alia, to the consolidation of short-term memories into long-term memories (Abraham et al., 2019). During motor learning, there is also the consolidation of motor memories into new sequences of muscle activity (Krakauer and Shadmehr, 2006; Dayan and Cohen, 2011). New knowledge and behaviors are recorded in the nervous system through changes in neural systems, and where each new piece of information reaching the nervous system triggers further changes (King et al., 2019). Thus, neuroplasticity is a process in which neural networks and synapses are rebuilt. This is the reason for the creation of an engram (memory trace), i.e., a structural or molecular change within a synapse or the entire neural network (Sakaguchi and Hayashi, 2012; Poo et al., 2016). Memory and learning are therefore not only behavioral phenomena but also reflect changes in the structure of the nervous system. Gene expression plays an important role in neuroplasticity, of particular importance is the brain derived neurotrophic factor (*BDNF*) gene (Ghassabian et al., 2017; Dorszewska et al., 2020).

The expression of specific genes can drive neuroplastic processes. In this regard, extensively studied are the polymorphisms of the *BDNF* gene (for example, Val66Met *BDNF* polymorphism), which encodes BDNF proteins secreted by neurons belonging to the nerve growth factor family. BDNF determines the functions of cholinergic and dopaminergic neurons and influences motor and sensory neurons. *BDNF* polymorphisms containing the Met allele are responsible for the conversion of valine (Val) to methionine (Met) at codon 66 (Val66Met), causing an approximately 25% reduction in BDNF protein activity and subsequently neuroplastic abilities (Lu et al., 2014; Park et al., 2017; McGregor and English, 2019). In this context, the executive molecule tropomyosin receptor kinase B (TrkB) should also be mentioned, as the interactions between BDNF and TrkB are very important in inducing dendrite growth (Cheung et al., 2007). This means that the activation of the BDNF-TrkB pathway plays an important role in learning and memory processes (Notaras and Van Den Buuse, 2019). Studies in mice have shown that the Val66Met *BDNF* polymorphism not only reduces BDNF expression but also epigenetically impairs TrkB expression (Ieraci et al., 2016). This confirms an even more significant relationship between *BDNF* genotypes and neuroplasticity capabilities. However, it should be noted that the expression of BDNF and TrkB and their interactions vary depending on the brain center (Klintsova et al., 2004).

Neuroplasticity is important in motor learning, therefore the relationship between *BDNF* genotypes and BDNF protein activity with neuroplasticity can help determine the effectiveness of motor rehabilitation. Current literature has shown the most beneficial genotype to be Val/Val, while Val/Met and Met/Met are associated with a decreased effectiveness of rehabilitation (Kim et al., 2016). These variants were studied in stroke patients and correlated with the progress of their rehabilitation and motor learning. A strong correlation was demonstrated between the presence of specific *BDNF* polymorphisms and the effects of rehabilitation in post-stroke patients. Confirming the important role of BDNF in neuronal plasticity (Kotlęga et al., 2017). A relationship was identified between the Val66Met *BDNF* polymorphism and the state of motor functions and the improvement after rehabilitation in chronic post-stroke patients (Shiner et al., 2016). It was also noted that subjects with the Met allele show reduced activity of the sensorimotor cortex (Kim et al., 2016). However, the activity of the BDNF protein depends not only on the genotype but is also intensified by motor activity. Therefore, both these factors play an important role in motor learning (Helm et al., 2017) by enhancing neuroplasticity in the M1 motor cortex (Fritsch et al., 2010). Moreover, the expression of BDNF during motor learning influences corticospinal excitability, even if the movement is passive (Van Pham et al.,

2021). The BDNF gene is also important in understanding differences in motor performance (González-Giraldo et al., 2014). In addition, the undertaken motor activity may affect the distribution and intensity of BDNF and TrkB protein responses (Skup et al., 2002). This shows that neuroplasticity in the context of motor learning depends not only on the genotype but also on the undertaken physical activity. The reorganization of functional nerve connections at the local and global level, induced by rehabilitation and supported by gene expression, contributes to the restoration and formation of normal function (McGregor and English, 2019).

METHODS

Research hypothesis

Motor rehabilitation is very important for maintaining and improving gait functions in children with CP. The expression of the *BDNF* gene, which is closely related to the plasticity of the nervous system, may help create memory traces responsible for new sequences of muscle activity. The results of many studies have found the Val/Val polymorphism to have the strongest positive correlation with the effectiveness of rehabilitation. Subjects with Val/Met and Met/Met polymorphisms showed significantly lower effectiveness of rehabilitation. Patients with CP are predicted, similarly to subjects after stroke, to show differences in the effectiveness of rehabilitation based on the genotype of *BDNF*. Participants with CP are predicted to show different successes with rehabilitation based on different *BDNF* genotypes. The improvement in gait parameters (GDI) is hypothesized to be greater in subjects with the Val/Val polymorphism. Functional improvements in standing and gait (Gross Motor Function Measure — Standing (GMFM-D) and GMFM — Walking, Running, and Jumping (GMFM-E)) are predicted to be greater in subjects with the Val/Val polymorphism. Additionally, the functional improvement in functional gait tests (6-Minute Walk Test (6MWT), 10-Minute Walk Test (10MWT), and Timed Up and Go Test (TUG)) is predicted to be greater in subjects with the Val/Val polymorphism. Our main hypothesis is that rehabilitation is more effective in CP patients with the Val/Val polymorphism compared to those with at least one Met allele.

Research tools and research method

This study was conducted in a group of children with CP who underwent rehabilitation at the Technology Supported Rehabilitation Center at the Orthopedic and Rehabilitation Clinical Hospital No. 4 of Poznan University of Medical Sciences. The study was approved by the Bioethics Committee of the Poznan University of Medical Sciences (resolution No. 245/20 of March 11, 2020), and participation in the study was voluntary. Parents or legal guardians gave their consent for the child's participation. The inclusion criterion included a diagnosis of CP and a functional evaluation classification using the Gross Motor Function Classification System (GMFCS) to include levels I–III. Participants were excluded from the study if they had undergone a recent surgery as it could significantly affect the quality of gait. In all subjects, the results of rehabilitation were compared with the results of genetic tests to examine the relationship between BDNF genotype and the effectiveness of rehabilitation (an indirect index of neuroplasticity).

The study group consisted of 50 subjects (F=25; M=25; GMFCS I–III), aged between 6 and 19 years (Me=11, SD=2.89, V=26.1%), undergoing rehabilitation with the use of robots and virtual reality. Despite the high variability in age, the data for children and adolescents have been combined because both groups completed the processes affecting the structural dynamics of neurons before any significant involutional changes have begun. Our research group allows us to study a wider age spectrum of people within the developmental ages. Moreover, this study did not use a functional scale that restrictively delineates certain age ranges.

Each patient had a swab taken to examine the *BDNF* genotypes. Swabs were taken from the inside of the cheek and each sample was given a number and collected using similar and systematic procedures. The samples were then stored at about -30°C for further analysis. DNA isolation was performed using column isolation kits according to the manufacturer's protocol. The kit used for isolation was the A&A Biotechnology SWAB kit (catalog number: 025–25). Isolation was completed using an RL lysis solution and proteinase K. The extraction was performed in an elution of Tris buffer (pH 8.5). All samples were analyzed together. The isolated DNA was sequenced using DNA strand melting analysis (HRM) and real-time PCR, and the obtained clusters allowed for the division of subjects into two groups. The VAL group was made up of subjects with homozygous Val/Val polymorphisms, and the MET group included patients with at least one Met allele (Val/Met (n=10) and Met/Met (n=4) polymorphisms).

After genetic analysis, the VAL group consisted of 36 subjects (F=16; M=20) aged 6 to 19 years (M=11.03, Me=11, SD=3.01, V=27.3%). The MET group consisted of 14 subjects (F=9; M=5) aged 7 to 15 years (M=11.21, Me=12, SD=2.67, V=23.8%). Detailed sequencing results are presented in Fig. 1. Demographic are presented is Table 1.

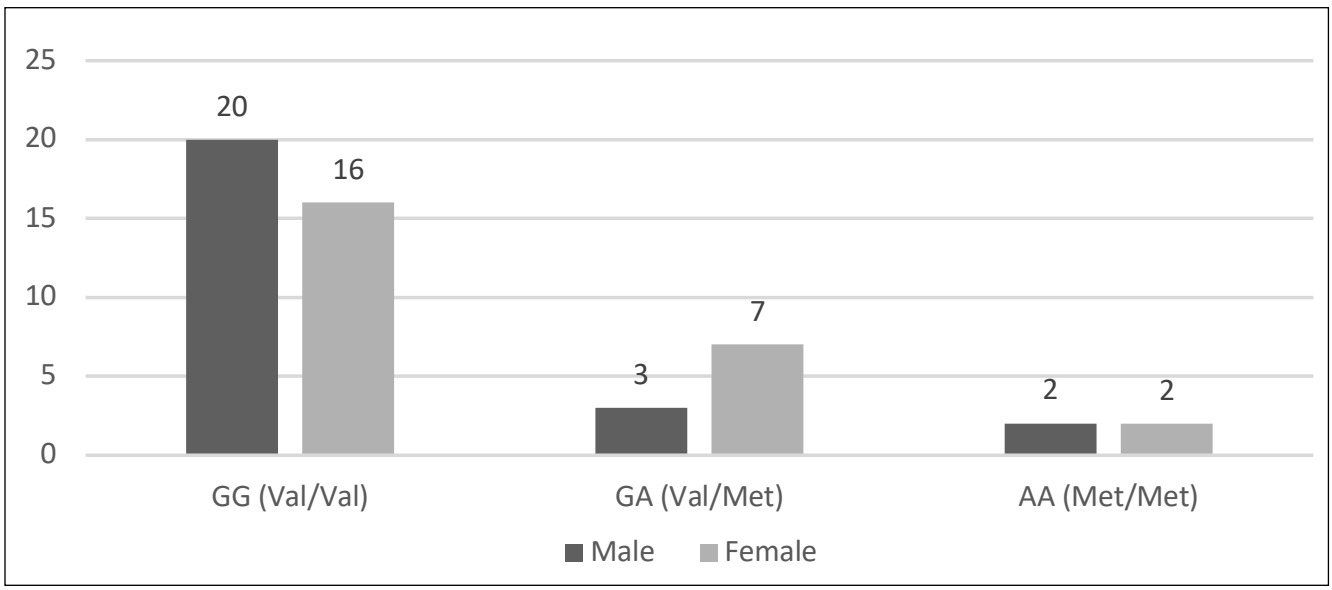

Fig. 1. A graph showing the distribution of genotypes in the studied sample and the distribution of sex in individual genotyping groups.

Table 1. Demographic table for the VAL and MET groups, taking into account the sex distribution, age profile, and functional status in the GMFCS classification. The numbers express the number of people and the percentages express what percentage of the entire VAL or MET group is in a given number of people. Difference between groups expressed in the number of people or percentage points (p.p.).

	VAL	MET	Difference
Total	36	14	22
Male	20 (56%)	5 (36%)	20 p.p.
Female	16 (44%)	9 (64%)	20 p.p.
Age 6–9	11 (31%)	4 (29%)	no significant
Age 10–14	15 (42%)	6 (43%)	no significant
Age 15–19	10 (27%)	4 (29%)	no significant
GMFCS I	12 (33%)	4 (29%)	no significant
GMFCS II	15 (42%)	7 (50%)	no significant
GMFCS III	9 (25%)	3 (21%)	no significant

Study participants underwent technology-assisted rehabilitation with the use of robots and virtual reality aiming to improve functional and motor skills in two-week cycles. During rehabilitation, the following devices were used: G-EO Evolution, Lokomat, Exoskeleton, and Zero-G. Appropriate loads were applied in a way to maximize the effects of the rehabilitation and the subject's independence. The main task of each device was to educate the gait pattern using a load progression method. In addition, the subjects underwent training on the Zebris treadmill focusing on the quality of gait (stride length, rhythm, speed) and hand-eye coordination as well as other treatment areas. The Alfa, Gamma, and Biodex dynamometric platforms used by the patients allowed for body balance training. The effectiveness of rehabilitation with the aforementioned devices has been repeatedly tested (Hidler et al., 2011; Kalron et al., 2013; Schroeder et al., 2014; Li et al., 2015; Riedo and Hunt, 2016), and is why they were chosen to provide a reliable and effective method of functional and motor rehabilitation. Rehabilitation took place for 2 weeks. Patients performed therapy exercises every day for 3.5–4 h from Monday to Friday.

To measure the functional and motor improvements of the participants in this study, a three-dimensional instrumental functional diagnostic test of gait was used. The Gait Deviation Index (GDI) was determined before the start of the 2-week therapeutic cycle. In addition, these functional tests were performed on the day the technology-assisted rehabilitation commenced: TUG, 10MWT, 6MWT, and the Gross Motor Function Measure (GMFM-88) scale, which is an observational clinical tool designed to evaluate changes in gross motor function in children with CP. However, due to the focus of rehabilitation being on gait education and re-education, only the elements of this scale related to standing (GMFM-D) and walking (GMFM-E) were used. The TUG measured the time in which it took participants to get up from a chair, walk 3 meters, turn, and sit on a chair again. The 10MWT measured the time it took the subjects to walk 10 meters. The 6MWT measured the distance that the subject was able to cov-

er in 6 min. All scales and tests used in this study have been standardized and are widely used (Meyer-Heim et al., 2009; Rasmussen et al., 2015; Carey et al., 2016; van Hedel et al., 2016). The use of these methods in our study allows it to provide reliable indicators of functional and motor improvements.

Instrumental gait examination was performed using the Vicon system (software: Nexus 2.12) with Motion-Capture technology (image capture) qualifying it as a passive optoelectronic measurement system. During the GDI assessment, the patient performed several passes, from which 3 representative passes were selected and the results were averaged. Functional assessments and rehabilitation were performed by qualified physiotherapists. Assessments before and after rehabilitation in a given patient were carried out by the same physiotherapist. The genetic test results did not affect the objectivity of the tests or the selection of rehabilitation parameters because the physiotherapists were blinded from the results before starting the collective data analysis. This analysis was performed by the entire research team.

The *BDNF* genotype was examined in all subjects and all measurements and tests were performed before and after rehabilitation. Immediately after rehabilitation, TUG, 10MWT, and 6MWT tests were performed. At an average of 6 months post-rehab, the GDI, GMFM-D, GMFM-E were remeasured and the TUG, 10MWT, and 6MWT were repeated. Increases in the values of the GDI, GMFM-D, GMFM-E, and 6MWT indicate functional and/or motor improvement, while the 10MWT and TUG tests require a decrease in the values to indicate functional improvement. The results of the molecular tests were compared to the functional test and gait parameter results once all participants completed the rehabilitation component. All rehab treatments were based on developing similar motor skills and functional patterns in each patient.

The following statistical tests were used: Shapiro-Wilk test (to test the normality of the distribution of variables in individual groups), Student's t-test for dependent variables (in the case of normal distribution; to compare the results before and after rehabilitation and separately for the VAL group and the MET group), Wilcoxon's test (in the absence of normal distribution; for the comparison of the results before and after rehabilitation and separately for the VAL group and the MET group), and the Mann-Whitney U-test (for the comparison of the groups in terms of improved scores). For the Student's t-test and Wilcoxon's test, a p-value of<0.05 was considered a statistically significant change in a group. For the Mann-Whitney U-test, a p-value of<0.05 was considered a statistically significant difference between groups. For statistically significant results, a power analysis was carried out taking into account the sample size, the effect level, and the direction of the hypothesis at an assumed significance level (α) of 0.05.

The statistical analysis was performed using the Statistica package (version 13.3).

RESULTS

Gait Deviation Index

The GDI improved in 69% of subjects in the VAL group, while in the MET group 50% saw improvement. In the VAL group, the GDI deteriorated in 31% of the subjects, and in the MET group, a decrease was observed in 50% of the subjects. The GDI values before rehabilitation and 6 months after rehabilitation for the VAL and MET groups, as well as the changes in the GDI values within individual groups and the individual statistical test results, are presented in Table 2.

Table 2. Summary of data obtained from measurements of the GDI coefficient in the VAL and MET groups. The GDI measurements are expressed in numbers (without units). The upper part of the table shows the GDI values in each group before and after rehabilitation, as well as, the Student's t-test results for dependent samples indicating whether the functional changes were statistically significant. The lower part of the table presents the changes in GDI coefficient values, the Mann-Whitney U-test results, and the power value to compare the improvement/deterioration in function between groups.

Gait Deviation Index (GDI)				
Group	VAL		MET	
Measurement	Before	After	Before	After
Min	52.63	52.41	65.47	68.20
Max	100.00	100.00	91.31	88.07
M	78.84	80.61	78.95	80.23
Me	80.32	84.27	78.88	79.93
SD	11.70	13.02	7.24	5.78
Shapiro-Wilk	p=0.89	p=0.54	p=0.17	p=0.26
t-Student	p=0.07		p=0.43	
Max. Improvement	+16.74		+11.23	
Max. Deterioration	-15.65		-10.47	
M	+3.43		-1.62	
Me	+0.91		-0.05	
SD	6.19		5.82	
U-Mann-W	p=0.011; 1-β≈0.85			

In both groups, the change in GDI turned out to be insignificant. The maximum improvement and a higher average improvement were observed in the VAL group, however, the highest decrease in GDI was also observed in this group. The difference between the medians of the group turned out to be statistically significant (p=0.011) towards the VAL group.

Gross Motor Function Measure — Standing

The GMFM-D index improved in 58% of subjects in the VAL group, while in the MET group 50% showed improvement. In the VAL group, there was a decrease in the GMFM-D index in 42% of the subjects, while in the MET group a decrease was observed in 50% of the subjects. The GMFM-D index values before rehabilitation and 6 months after rehabilitation for the VAL and MET groups, as well as the changes in the GMFM-D index values within individual groups and the individual statistical test results are presented in Table 3.

Table 3. Summary of data obtained from measurements of the GMFM-D coefficient in the VAL and MET groups. The GMFM-D measurements are expressed in numbers (without units). The upper part of the table shows the GMFM-D values in each group before and after rehabilitation, as well as, Wilcoxon's test results indicating whether the functional changes were statistically significant. The lower part of the table presents the changes in GMFM-D coefficient values and the Mann-Whitney U-test results to compare the improvement/deterioration in function between groups.

GMFM — Standing (GMFM-D)				
Group	VAL		MET	
Measurement	Before	After	Before	After
Min	25.60	25.60	5.10	5.10
Max	100.00	100.00	100.00	100.00
M	83.43	83.56	75.98	77.62
Me	90.85	89.70	83.31	87.16
SD	18.09	16.54	28.05	30.11
Shapiro-Wilk	p=0.00	p=0.00	p=0.001	p=0.00
Wilcoxon	p=0.91		p=0.39	
Max. Growth	+20.54		+15.40	
Max. Decline	-10.37		-10.21	
M	+0.69		+0.55	
Me	+0.00		+0.22	
SD	7.33		7.57	
U-Mann-W	p=0.99			

In both groups, the change in the GMFM-D index turned out to be insignificant. The maximum improvement and a higher average improvement were observed in the VAL group, however, the highest decrease in the GMFM-D index was also observed in this group. The highest median of improvement was observed in the MET group. However, the difference in medians between the groups was not statistically significant (p=0.99).

Gross Motor Function Measure — Walking, Running, and Jumping

The GMFM-E index improved in 69% of subjects in the VAL group, while in the MET group 64% showed improvement and was maintained in 22% of them. In the VAL group, the GMFM-E index decreased in 31% of the subjects, while in the MET group a decrease was observed in 36% of the subjects, and the downward trend continued in 75%. The GMFM-E index values be-

Table 4. Summary of data obtained from measurements of the GMFM-E coefficient in the VAL and MET groups. The GMFM-E measurements are expressed in numbers (without units). The upper part of the table shows the GMFM-E values in each group before and after rehabilitation, as well as the Wilcoxon's test results indicating whether the functional changes were statistically significant. The lower part of the table presents the changes in GMFM-E coefficient values, the Mann-Whitney U-test results, and the power value to compare the improvement/deterioration in function between groups.

GMFM — Walking, Running and Jumping (GMFM-E)				
Group	VAL		MET	
Measurement	Before	After	Before	After
Min	8.33	8.33	0.00	1.40
Max	100.00	100.00	98.60	100.00
M	73.86	76.08	71.13	70.26
Me	84.85	87.50	84.02	79.17
SD	27.14	26.67	33.27	32.75
Shapiro-Wilk	p=0.00	p=0.00	p=0.002	p=0.006
Wilcoxon	p=0.63		p=0.03	
Max. Growth	+20.82		+6.93	
Max. Decline	-9.72		-20.80	
M	+3.22		-2.61	
Me	+0.00		+0.00	
SD	6.03		7.33	
U-Mann-W	p=0.01; 1-β≈0.88			

fore rehabilitation and 6 months after rehabilitation for the VAL and MET groups, as well as the changes in the GMFM-E index values within individual groups and the individual statistical test results, are presented in Table 4.

In the MET group, the change in GMFM-E index was significant, but in the VAL group, the change did not reach statistical significance. There was no difference between the groups in terms of median improvement. The maximum improvement and a higher mean improvement were observed in the VAL group, while the highest deterioration was observed in the MET group. The difference between groups turned out to be statistically significant (p=0.01), but there was no difference between the medians. After comparing another measure of central tendency (M), the difference was found to be statistically significant in favor of the VAL group.

6-Minute Walk Test

The 6MWT index improved in 69% of subjects in the VAL group and the improvement was maintained in 44% of them. The MET group observed an improvement in 79% of subjects that was maintained in 36% of them. In the VAL group, there was a decrease in 31% of subjects, and the downward trend was maintained in 27% of them. The MET group observed a decrease in 21% of the subjects that was maintained in 67% of them. The 6MWT index values before and immediately after rehabilitation for the VAL and MET groups, as well as the changes in the 6MWT index values within individual groups and the individual statistical test results are presented in Table 5.

In both groups, the changes in the 6MWT index were statistically significant. The highest improvement and a higher mean improvement were observed in the VAL group, while the highest decrease was observed in the MET group. A higher average improvement was observed in the MET group. The difference between the medians of the groups was statistically significant (p=0.009) favoring the MET group.

10-Meter Walk Test

The 10MWT index improved (a decrease in value) in 58% of subjects in the VAL group and the improvement was maintained in 71% of them. The MET group observed improvements in 79% of participants. In the VAL group, there was a deterioration (increase in value) in 42% of the respondents and the trend was maintained in 24% of them. The MET group, observed deterioration in 21% of subjects. The values of the 10MWT index before and immediately after rehabilitation for the VAL and MET groups, as well as the changes in the 10MWT index values and the individual statistical test results are presented in Table 6.

In the VAL group, the change in the 10MWT index was statistically significant, while the MET group failed to reach statistical significance. The maximum improvement and a higher average improvement were observed in the MET group, and the greatest deterioration was observed in the VAL group. The difference between the medians of the groups turned out to be statistically significant (p=0.048) towards the MET group.

Timed Up and Go Test

The TUG index improved (decrease in value) in 69% of subjects in the VAL group, and the improvement was

Table 5. Summary of data obtained from measurements of the 6MWT coefficient in the VAL and MET groups. The 6MWT measurements are expressed in meters. The upper part of the table shows the 6MWT values in each group before and after rehabilitation, as well as the Student's t-test results for dependent samples, the Wilcoxon's test results depending on the normality of the distribution, and the power values indicating whether the functional changes were statistically significant. The lower part of the table presents the change in 6MWT coefficient values, the Mann-Whitney U-test results, and the power value to compare the improvement/deterioration in function between groups.

6-Minute Walk Test (6MWT)				
Group	VAL		MET	
Measurement	Before	After	Before	After
Min	30.00	48.00	29.00	50.00
Max	519.00	610.00	559.00	606.00
M	344.58	374.64	322.00	362.29
Me	380.00	396.00	343.50	384.00
SD	119.58	122.40	151.22	146.21
Shapiro-Wilk	p=0.002	p=0.027	p=0.70	p=0.52
t-Student/ Wilcoxon	(Wilcoxon) p=0.00 1-β≈0.28		(t-Student) p=0.035 1-β≈0.18	
Max. Growth	+152.00		+69.00	
Max. Decline	-45.00		-81.00	
M	+56.91		+14.14	
Me	+23.00		+46.00	
SD	46.84		40.06	
U-Mann-W	p=0.009; 1-β≈0.94			

Table 6. Summary of data from measurements of the 10MWT index in the VAL and MET groups. The 10MWT measurements are expressed in seconds. The upper part of the table shows the 10MWT values in each group before and after rehabilitation, as well as the Wilcoxon's test results indicating whether the functional changes were statistically significant. The lower part of the table presents the change in 10MWT index values, the Mann-Whitney U-test results, and the power value to compare the improvement/deterioration in function between groups.

10-Meter Walk Test (10MWT)				
Group	VAL		MET	
Measurement	Before	After	Before	After
Min	6.10	6.01	6.24	4.79
Max	118.00	104.00	145.00	75.00
M	15.39	14.23	22.57	14.75
Me	9.67	9.32	10.46	9.69
SD	20.36	18.63	36.72	17.89
Shapiro-Wilk	p=0.00	p=0.00	p=0.00	p=0.00
Wilcoxon	p=0.046		p=0.14	
Max. Improvement	-15.18		-70.00	
Max. Deterioration	+1.14		+1.05	
M	-2.42		-5.62	
Me	-0.84		-0.87	
SD	3.69		18.62	
U-Mann-W	p=0.048; 1-β≈0.27			

Table 7. Summary of data obtained from measurements of the TUG index in the VAL and MET groups. The TUG measurements are expressed in seconds. The upper part of the table shows the TUG values in each group before and after rehabilitation, as well as the Wilcoxon's test results indicating whether the functional change was statistically significant. The lower part of the table presents the changes in TUG index values, the Mann-Whitney U-test results, and the power value to compare the improvement/deterioration in function between groups.

Timed Up and Go Test (TUG)				
Group	VAL		MET	
Measurement	Before	After	Before	After
Min	6.40	5.79	6.18	5.11
Max	128.00	90.60	105.00	48.79
M	17.70	14.08	18.92	12.68
Me	10.38	9.30	10.62	8.92
SD	26.98	17.19	26.21	11.62
Shapiro-Wilk	p=0.00	p=0.00	p=0.00	p=0.00
Wilcoxon	p=0.04		p=0.15	
Max. Improvement	-55.00		-17.00	
Max. Deterioration	+0.51		+0.12	
M	-4.66		-2.62	
Me	-1.41		-1.25	
SD	10.20		4.72	
U-Mann-W	p=0.039; 1-β≈0.25			

maintained in 35% of them. The MET group observed improvements in 93% of subjects. In the VAL group, there was a deterioration (increase in value) of the TUG index in 31% of the subjects, and the trend was maintained in 36% of them. The MET group observed a deterioration in 7% of subjects. The values of the TUG index before and immediately after rehabilitation for the VAL and MET groups, as well as the changes in the TUG index values and individual statistical test results are presented in Table 7.

In the VAL group, the change in the TUG index was statistically significant, while in the MET group did not reach statistical significance. The maximum improvement, a higher average improvement, and a higher mean improvement were observed in the VAL group, however, the greatest deterioration was also observed in this group. The difference between the medians of the groups turned out to be statistically significant (p=0.039) towards the VAL group.

DISCUSSION

Although improvements in the test parameters were significant in only a few cases (GMFM-E for the MET group; 6MWT for both groups; 10MWT and TUG for the VAL group), the differences between the VAL and MET groups were statistically significant for all indicators, except for GMFM-D. The differences between groups in regards to improvements on the 6MWT and 10MWT indicators showed the opposite tendency than was expected. This study was able to answer the research hypotheses. The differences between the groups were statistically significant in 5 out of 6 measured indicators. The improvement in the GDI was greater and reached statistical significance in subjects with the Val/Val genotype. The improvement in the GMFM-D index was similar in both groups, however, the improvement in the GMFM-E index was statistically significantly higher in subjects with the Val/Val genotype. The improvement

in the 6MWT and 10MWT indices was statistically significantly higher in subjects with the Met allele, while the improvement in the TUG index was statistically significantly higher in those with the Val/Val genotype.

After analyzing the molecular, clinical, and biometric data, it was found that the better outcomes in the VAL group were in improving gait pattern quality (GDI), the functional level index for locomotion and other dynamic functions (GMFM-E), as well as complex motor activity measurement (TUG). These results may indicate that subjects with the Val/Val genotype are predisposed to achieve more significant functional improvements. Maintaining the improvements despite the time lapse between GDI and GMFM-E measurements may indirectly indicate the creation of a stronger memory trace for new motor sequences.

The better results in the MET group occurred on tests indirectly examining the quality of gait and were greatest in tests where speed of task performance played the most important role (6MWT and 10MWT). This is in line with the results of another study that showed carriers of the Met allele achieved faster response times during task performance and achieved higher task speeds (Baird et al., 2018).

The lack of a significant difference between the groups in the standing functional level index (GMFM-D) may indicate that BDNF supports motor learning related to the education of dynamic patterns and not static stability.

This study does not allow for a clear statement that the *BDNF* genotype is associated with greater functional or motor improvement. The difference between the VAL and MET groups differed not only by genotype but also in sex distribution as 56% of men were in the VAL group and only 36% in the MET group. This study failed to take into account other genes that could be important in determining plasticity of the nervous system and cognitive flexibility, such as *SRGAP2* (Tsai, 2018) or *COMT* (Nogueira et al., 2020) or genes related to the pathogenesis of CP — *MTHFR* (Cheng et al., 2011). In addition, epigenetic factors that could modernize *BDNF* activity (Nooshabadi et al., 2016), other epigenetic factors of importance in CP (Crowgey et al., 2018; Mohandas et al., 2018), and prematurity-associated risks of neurodevelopmental disorders (Blair et al., 2016) were not taken into account.

The diversity of genotypes and significant advantage of the "favorable" Val/Val genotype among subjects, allows one to assume that in the case of CP, unlike in the case of stroke (Balkaya and Cho, 2019), the *BDNF* genotype does not predict an increased risk of CP pathogenesis. In other studies (Trevarrow et al., 2018), as in the presented study, a better functional improvement was found in subjects with the Val/Val genotype than in subjects with the Met allele. Long-term prognosis related to functional mobility, however, should not be based only on the *BDNF* genotype (French et al. 2018).

CONCLUSION

In this study, results were not reported by GMFCS levels because the sample size was too small to make such a division. Moreover, the aim of the study was primarily to investigate the correlation between genotypes and the improvement in specific kinematic and functional parameters. Therefore, the tests were also not relativized or adjusted to GMFCS level. A study with a larger number of subjects, equal groups, and analysis of a greater number of polymorphisms, as well as taking into account other factors (e.g., prematurity or functional division according to GMFCS classification) is needed. This would allow for a more detailed examination of the potential relationship of genetic factors on the effects of motor learning which could lead to the identification of genetic and epigenetic biomarkers in the rehabilitation of CP patients at different functional levels according to the GMFCS classification.

Genetic polymorphisms may affect the ability of subjects with CP to exercise neuroplasticity and affect their sensitivity to neurological rehabilitation. Understanding genetic variation can be used to predict the chances of restoring or reshaping function and design an optimal treatment regimen. Characterizing the relationship between genes and the response to rehabilitation can be an important step towards personalized medicine and as a potential prognostic tool.

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