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Research Paper

Genetic Variation in the Dopamine System Influences Intervention Outcome in Children with Cerebral Palsy

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ABSTRACT

Background: There is large variation in treatment responses in children with cerebral palsy. Experimental and clinical results suggest that dopamine neurotransmission and brain-derived neurotrophic factor (BDNF) signalling are involved in motor learning and plasticity, which are key factors in modern habilitation success. We examined whether naturally occurring variations in dopamine and BDNF genes influenced the treatment outcomes.
Methods: Thirty-three children (18–60 months of age) with spastic unilateral cerebral palsy were enrolled in the study. Each child had participated in a training programme consisting of active training of the involved hand for 2 h every day during a 2-month training period. The training outcome was measured using Assisting Hand Assessment before and after the training period. Saliva was collected for genotyping of COMT, DAT, DRD1, DRD2, DRD3, and BDNF. Regression analyses were used to examine associations between genetic variation and training outcome.
Findings: There was a statistically significant association between variation in dopamine genes and treatment outcome. Children with a high polygenic dopamine gene score including polymorphisms of five dopamine genes (COMT, DAT, DRD1, DRD2, and DRD3), and reflecting higher endogenous dopaminergic neurotransmission, had the greatest functional outcome gains after intervention.
Interpretation: Naturally occurring genetic variation in the dopamine system can influence treatment outcomes in children with cerebral palsy. A polygenic dopamine score might be valid for treatment outcome prediction and for designing individually tailored interventions for children with cerebral palsy.

1. Introduction

A plethora of experience-derived treatments for cerebral palsy have been developed over time. Researchers and clinicians have only during the past few decades adopted an evidence-based approach to identify and use effective interventions (Novak et al., 2013). New therapies based on active motor learning and motor training have been found to improve motor function and activity (e.g., modified constraint-induced movement therapy, bimanual training, and goal-directed training) (Eliasson et al., 2005; Eliasson et al., 2011; Gordon et al., 2007; Ketelaar et al., 2001). A common problem with these studies is that they focus on main effects at the group level, but neglect the effects of individual differences (Damiano, 2014). Due to large inter-individual variation in treatment response, there are often barely significant differences between the study groups (see Figs. 2 and 4 in (Chiu and Ada, 2016) and Fig. 3 in (Eliasson et al., 2005)). An intervention that is effective for one child with cerebral palsy may not be effective for another child.

These observed large inter-individual differences in treatment outcomes have negative consequences for non-responders because time and effort will be expended without any gains in functional improvement. Identification of the causes of the variability is an important step in the advancement of personalized rehabilitation medicine. Each child with cerebral palsy can then receive an individually tailored intervention. The concept of personalized medicine has evolved mainly from the variability observed in response to various drugs. It has developed into the field of pharmacogenetics, which examines how genetic differences (especially in metabolic pathways) affect individual responses to drugs (Roses, 2000). The genetic effects may not be as influential for therapeutic interventions in children with cerebral palsy. However, identification of factors that predict the individual's response to an intervention would be useful for the patient and for the health providers attempting to optimize care.

Several factors may influence the outcome of interventions in cerebral palsy, which encompasses heterogeneous clinical phenotypes and aetiologies. Factors that can affect the outcome of an intervention can be associated with the white and/or grey matter brain injuries of

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varying location, size, and time of origin. These conditions are known to affect cognitive and motor functions. Many children have no detectable risk factors, and 30% of the cases of cerebral palsy may be of genetic origin (Fahey et al., 2017). However, the contribution of genetic variation to treatment outcomes remains mostly unknown.

Functional genetic variation can influence motor learning and the cortical plasticity that form the principle foundation for modern rehabilitation interventions. One of the best-characterized examples is the functional val^{66}met polymorphism in the gene for brain derived neurotrophic factor (BDNF). BDNF is highly expressed throughout the brain and has important roles in development, plasticity, and repair. The presence of the BDNF val^{66}met polymorphism is associated with poor short-term motor learning gains and altered short-term cortical plasticity (McHughen et al., 2010). Results of animal studies indicate that microglial BDNF has an important physiological function in motor learning via promotion of learning-related synapse formation (Parkhurst et al., 2013).

Dopamine signalling is an essential component of various brain functions (e.g., motor control, reward, learning, and plasticity) (Chudasama and Robbins, 2006). Rodent studies have found that mesocortical dopaminergic pathways from the ventral tegmental area to the motor cortex are involved in skilled motor learning and associated synaptic plasticity (Molina-Luna et al., 2009). This result suggests that the motor cortex requires an optimal level of dopamine for learning new motor skills The role of dopamine signalling is further corroborated by the association between acquisition of new motor skills and changes in the intracellular cAMP/PKA/DARPP-32 pathway; inhibition of this pathway impairs motor learning (Qian et al., 2015). A naturally occurring genetic variation in the rodent mesocortical dopamine system parallels differences in motor skill learning and plasticity (Qian et al., 2013). Results of human studies indicate that functional polymorphisms in genes encoding for dopamine receptors, and dopamine transporter and degradation enzymes, contribute to inter-individual differences in learning and cognitive performance. Polymorphisms that reduce dopamine transmission are associated with poorer function (Pearson-Fuhrhop et al., 2013; Baetu et al., 2015; Noohi et al., 2016; Noohi et al., 2014; Huertas et al., 2012). Pearson-Fuhrhop et al. (2013) found that genetic variation in the human dopamine system affects motor learning outcomes in healthy adults. A gene score reflecting the collective effects of five dopamine polymorphisms associated with synaptic dopamine availability (COMT and DAT) and dopamine receptor binding (DRD1, DRD2, DRD3) was an important contribution of this study. The authors found that individuals with the higher dopamine scores that corresponded to higher dopaminergic neurotransmission also had significantly greater motor learning rates.

The results of human and animal studies thus suggest that dopamine and BDNF are involved in motor learning, and that variation in dopamine and BDNF genes might contribute to the inter-individual differences in treatment response. The aim of this study was to examine the influences of functional genetic variation in the dopamine system and BDNF on the outcome of an intervention programme for children with cerebral palsy. The intervention used was modified Constraint Movement Therapy (CIMT) (Eliasson et al., 2005; Eliasson et al., 2011), which is based on active motor learning and motor training.

2. Methods

2.1. Participants

The participants were recruited from two previous intervention studies of children with spastic unilateral cerebral palsy. The first study was a controlled clinical trial that included 21 children (18––48 months of age) (Eliasson et al., 2005). The second study used a randomized crossover design that included 25 children (18–60 months of age) (Eliasson et al., 2011). Each child underwent an Assisting Hand Assessment (AHA) before and after the intervention and had complied with the scheduled training program. Each of the 46 children was invited to participate in this study 6–15 years after the first two trials, when they were asked to provide a saliva sample for genetic analysis. Thirty-five subjects accepted the invitation. The age, sex, and AHA-unit at baseline or after training characteristics did not differ between those who accepted versus those who did not respond to, or refused, the invitation to participate ($n = 11$).

Each participant provided written informed consent prior to the collection of the saliva sample. The study was approved by the Regional Ethical Review Board in Stockholm (Dnr: 2015/61–31/2).

2.2. Intervention

The training programme for both studies comprised active training of the involved hand for 2 h each day during a 2-month training period. Toys and activities relevant for the age and ability of the child were used. During this modified Constraint-Induced Movement Therapy (CIMT), a comfortable fabric glove with a built in volar stiff plastic splint was worn on the less impaired hand to encourage each child to use the impaired hand (Eliasson et al., 2005; Eliasson et al., 2011). Parents and school teachers acted as treatment providers after attending an introductory educational and training session. One therapy session per week was supervised by a therapist. The results of each training session were recorded in a log book.

2.3. Assessment

The change in the Assisting Hand Assessment (AHA) (Krumlinde-Sundholm et al., 2007; Holmefur et al., 2007; Holmefur and Krumlinde-Sundholm, 2016; Holmefur et al., 2009) performed before and after the intervention period was used as the primary outcome of the CIMT intervention. Each assessment consisted of a 15-min long, semi-structured, video-recorded play session with toys requiring bimanual manipulation. The bimanual activity was scored for 22 items using a 4-point rating scale. The raw scores were converted to logits using Rasch analysis and transformed to a 0- to 100-unit scale (Holmefur et al., 2009). Each video recording was scored by a blinded evaluator who did not know the children, group allocation, or time of assessment (before versus after intervention).

In this study, we used the change in AHA-units (i.e., after CIMT minus before CIMT) as the primary outcome variable to examine the effect of genetic variation on intervention outcome.

2.4. Genetic Analysis

Saliva samples were collected from 35 children with spastic unilateral cerebral palsy using the Oragene DNA Sample Collection Kit (Genotek, Ottawa, Ontario, Canada). DNA was extracted within 3 days of collection following the manufacturer's protocol. DNA quantity and quality were evaluated using a NanoDrop TM 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Data from 33 participants were included in the analysis because two poor-quality saliva DNA samples were excluded. All samples were stored at −20 °C until use. Genotype was determined for COMT rs4680, DRD1 rs4532, DRD2/ANKK1 rs1800497, DRD3 rs6280, DAT1 VNTR, and BDNF rs6265 using polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis as previously described (Pearson-Fuhrhop et al., 2013). All saliva DNA samples were genotyped by an investigator blinded to the subjects' identity.

A combined “polygenic” dopamine gene score was determined as previously described (Pearson-Fuhrhop et al., 2013). Briefly, this score represented the cumulative effects of five dopamine-related polymorphisms with established biological functional effects on dopamine neurotransmission. Each genotype associated with low dopamine signalling received a score of zero; each genotype with a high signal received a score of one. The sum of the five individual genotypes ranged from

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