Candidate Genes Associated with the Course of Epilepsy in Cerebral Palsy: Remission or Refractoriness

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Abstract: Epilepsy often accompanies congenital cerebral palsy (CP). Canalopathies can be the cause of congenital epilepsy. The aim of the study is to determine the influence of various determinants on the course of epilepsy. Materials and methods: The results of clinical and genetic analysis of 136 cases of cerebral palsy (CP) with epilepsy are presented. The patients were divided into groups according to the syndromes according to the classification of CP (Panteliadis and R. Korinthenberg, 2005). Epileptic syndromes were divided into three groups: focal childhood epilepsy with structural brain changes and benign epileptiform discharges (BEDC) in EEG - 41 children (30.1%), structural focal epilepsy - 37 children (27.2%), epileptic encephalopathies 58 children (42.7%). Pathogenic variants in genes were confirmed by next generation sequencing (NGS) Sanger methods of venous blood. Results. Remission was more difficult to achieve in patients with determinants of regulation of general aspects of cellular metabolism, mitochondrial function, cytoskeleton formation and function, and transport across the outer membrane. The need for polypharmacy was in the groups that regulate the function of mitochondria, the formation and functioning of the cytoskeleton, and the regulation of membrane excitability. Conclusion. Determinant analysis provides a better understanding of the mechanisms of patient responsiveness to anticonvulsant therapy. The determinant of mitochondrial function most significantly affects its effectiveness. Probably, the violation of energy metabolism in the cell neutralizes the stabilization of the neuronal membrane under the influence of anticonvulsants. The determinant of the formation and functioning of the cytoskeleton, according to our preliminary data, is associated with the formation of malformations of the brain. In this case, the refractoriness of epilepsy can be secondary and determined by the severity of structural changes in the brain.

Keywords: perinatal brain lesion; cerebral palsy; epilepsy; genetics; pharmacogenetics; anti-epileptic drugs; anticonvulsants.

Introduction

The problem of congenital cerebral palsy does not lose its relevance. The reason is the severity of movement and mental disorders and not decreasing (at least) the number of cases of the disease.

In the phenotype of cerebral palsy, motor and mental disorders are often accompanied by epilepsy. Congenital epilepsy has been intensively researched in recent years.
Special attention is drawn to epilepsy caused by congenital disturbance of the excitability of the neuronal membrane due to canalopathies [1, 2, 3].

Traditionally, the causes of the development of the disease are considered to be hypoxia-ischemia, intoxication of the mother and fetus, and natal trauma. However, about 30% of cases of CP cannot be explained by the influence of these factors. Therefore, in recent years, genetic research has been intensively carried out [4].

The number of genes associated with the development of the cerebral palsy phenotype is constantly growing. Attempts have already been made to classify these genes according to the determinable enzyme and the process in the functioning of the cell. Thus, McMichael, Bainbridge and co. identified such directions of action of associated genes as axon navigation, participation in protein intrasynaptic interactions, and participation in synaptic transmission [5].

**The aim of the study**

Analyze a large number of genes associated with the development of the CP phenotype and distribute them according to determinable traits.

**Materials and Methods**

We present the results of a study of 373 cases of cerebral palsy with epilepsy in children aged 1 to 17 years. In 136 of them (36.5%), gene abnormalities were detected by the NGS method. The patients were divided into groups according to the syndromes according to the classification of CP (Panteliadis and R. Korinthenberg, 2005) spastic CP - 45 children (40.4%), dyskinetic and hyperkinetic forms of CP - 31 children (27.2%), ataxia with hypotonia - 50 children (36.8%).

Epileptic syndromes are divided into three groups: focal childhood epilepsy with structural brain changes and BEDC (benign epileptiform discharges) in EEG - 41 children (30.1%), structural focal epilepsy - 37 children (27.2%), epileptic encephalopathies (EE) 58 children (42.7%).

Patients underwent a routine examination with fixation of neurological symptoms, and the degree of impairment of large motor functions was assessed according to the GMFSC (Gross Motor Function Classification System) scale. Diagnosis of types of epileptic seizures, forms of epilepsy and epileptic syndromes was based on the classification of electro-clinical syndromes and other forms of epilepsy presented by the International League Against Epilepsy (ILAE) Operational classification of seizure types 2017 and the Classification of the Epilepsies 2017.

All patients were examined by video monitoring EEG (including video monitoring EEG of sleep) and magnetic resonance imaging (MRI). Electrophysiological studies were carried by EEGA-21/26 "ENTSEFALAN-131-03", modification 11, by Medicom MTD, Russia; "Neuroscope 6.1.508", Biola, Russia.

Pathogenic variants in genes were confirmed by NGS methods. The patients’ venous blood was examined. Deoxyribonucleic acid (DNA) isolation was carried out using a QIAGEN reagent kit (USA) in accordance with the manufacturer’s protocol. Mass parallel sequencing was performed using an Illumina NextSeq500 sequencer.

Data processing was carried out according to a proprietary algorithm, which includes alignment to a reference sequence, colling and annotation of variants. The determination of the clinical significance of the variants was carried out taking into account the ACMG (American College of Medical Genetics and Genomics) recommendations and the correspondence of the patient’s phenotype to the signs of the disease associated with the gene in which the pathogenic variant was found.

The distribution of genes into groups was carried out according to the classification of P.L. Sokolov, N.V. Chebanenko and co-authors (2020) [6].
Results and Discussion

We analyzed a large number of genes associated with the development of the CP phenotype and distributed them according to determinable signs.

We got 13 groups:
1. General aspects of the regulation of cell metabolism. (General Aspects Cell Metabolism - GACM).
2. Regulation of processes, the disorder of which leads to the formation of storage diseases (Group Storage Diseases - GSD).
3. Regulation of mitochondrial function in addition to the functional of group 2 (Regulation Mitochondrial Function - RMF).
4. Regulation of cell tolerance to external influences (hypoxia, ischemia, exogenous intoxication, etc.) (Cell Tolerance - CT).
5. Regulation of the formation and functioning of the cytoskeleton (CytoSceleton - CS).
6. Regulation of neuroontogenesis (neuronal migration, sprouting, synaptogenesis, myelination and apoptosis) (NeuroOntoGenesis - NOG).
7. Regulation of intracellular transport and secretion (functioning of the Golgi complex) (Golgi Complex - GC).
8. Regulation of transport across the external membrane of the cell. (External Cell Membrane - ECM).
9. Regulation of the excitability of the neuronal membrane (function of ion channels) (Excitability of Neuronal Membrane - ENM).
10. Regulation of ribosomal protein synthesis (Ribosomal Protein Synthesis RPS).
11. Regulation of the exchange of neurotransmitters and the functioning of synapses (NeuroTransmitters Synapses - NTS).
12. Regulation of immunity and oncogenesis (Immunity OncoGenesis - IOG).
13. Control of chromatin modifications, transcription and replication processes (Chromatin Modifications Transcription Replication - CMTR).

Genes, combined into groups GASM, GSD and RMF, determine cellular metabolism, groups CS and NOG - processes associated with neuroontogenesis, ENM, RPS and NTS - processes of intracellular secretion and membrane transport.

In the case of multifunctionality, the gene is classified according to the principle of the greatest influence on the phenotype.

We investigated the dependence of the refractoriness of epilepsy on the processes of neuron functioning determined by abnormal genes.

For convenience, we have divided the tables and in one presented the data in absolute figures, and in the other - in relative, in percentages (table 1).

Table 1. The degree of refractoriness of epilepsy in 136 patients with congenital cerebral palsy.

<table>
<thead>
<tr>
<th>Group of genes</th>
<th>Clinical remission</th>
<th>Neurophysiological remission</th>
<th>Clinical and neurophysiological remission</th>
<th>Reducing seizures by 50%</th>
<th>Lack of remission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute numbers</td>
<td>Relative data in percent</td>
<td>Absolute numbers</td>
<td>Relative data in percent</td>
<td>Absolute numbers</td>
</tr>
<tr>
<td>GASM</td>
<td>2</td>
<td>33</td>
<td>1</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>GSD</td>
<td>5</td>
<td>42</td>
<td>1</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>RMF</td>
<td>1</td>
<td>25</td>
<td>2</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>7</td>
<td>39</td>
<td>2</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>
53 of 136 patients in the general cohort had the absence of seizures. In 21 out of 136 patients (15.4%), the frequency of seizures was halved. But 50 patients (36.7%) failed to achieve remission. Complete remission was achieved only in 9 cases out of 138 (6.5%) (table1).

Relative numbers showed us that the most resistant to the treatment were patients with gene abnormalities from groups GASM (general aspects of the regulation of metabolism in the cell), RMF (regulation of mitochondrial function), CS (regulation of the formation and function of the cytoskeleton), and ECM (regulation of transport through outer membrane of the cell).

It was possible to achieve remission in any expression in all patients of groups CT (regulation of cell tolerance to external influences (hypoxia, ischemia, exogenous intoxication, etc.), NOG (regulation of neuroontogenesis), RPS (regulation of ribosomal protein synthesis) and IOG (regulation immunity and oncogenesis).

The prognosis was most favorable in patients whose phenotype is associated with genes of group NOG (regulation of neuroontogenesis).

Of interest is the fact that patients with a phenotype associated with canalopathy genes (ENM group) had remission in more than two thirds of cases. That is, in this group, the epileptic process was not the most refractory and not the most aggressive.

We also analyzed the therapeutic tactics used in our patients.

The number of antiepileptic drugs was analyzed depending on the groups to which the genes associated with the phenotype belonged. In the general cohort, the number of patients taking one anticonvulsant, two anticonvulsants, and three or more did not differ significantly (Table 2).

Table 2. The number of antiepileptic drugs in the treatment of epilepsy in 136 patients with congenital cerebral palsy.
In all three cases associated with group CT genes (regulation of cytoskeleton formation and function) polypharmacy was not required.

The largest proportion of patients receiving three or more anticonvulsants was in groups RMF (regulation of mitochondrial function), CS (regulation of the formation and functioning of the cytoskeleton), and ENM (regulation of neuronal membrane excitability) (table 2).

The epileptic process in congenital cerebral palsy is refractory to therapy. According to our data, it is possible to achieve complete remission only in 9 (6.5%) cases.

Refractoriness and the need for polypharmacy have a different map of determinants.

It was easier to achieve remission in patients with determinants of regulation of cell tolerance to external influences, neuroontogenesis, ribosomal protein synthesis, as well as immunity and oncogenesis.

Remission was more difficult to achieve in patients with determinants of regulation of general aspects of cell metabolism [7], mitochondrial function, formation and function of the cytoskeleton [8], as well as transport across the external membrane of the cell [9].

At the same time, the need for three or more anticonvulsants was in the groups that regulate the function of mitochondria, the formation and functioning of the cytoskeleton, and the regulation of the excitability of the neuronal membrane.

We assume that the determinant of mitochondrial function most significantly affects the effectiveness of anticonvulsant therapy.

Probably, the disorder of energy metabolism in the cell neutralizes the stabilization of the neuronal membrane under the influence of anticonvulsants [10]. This is also evidenced by the great need of patients of this group for polypharmacy. Polypharmacy affects several mechanisms of the excitability of the neuronal membrane at once and increases the effectiveness of therapy in these difficult cases.

The determinant of the formation and functioning of the cytoskeleton, according to our preliminary data, is largely associated with the formation of cerebral malformations. In this case, the refractoriness of epilepsy may be secondary and determined by the severity of structural changes in the brain [11].

**Conclusions**

Remission was more difficult to achieve in patients with determinants of regulation of general aspects of cell metabolism, mitochondrial function, formation and function of the cytoskeleton, as well as transport across the external membrane of the cell. At the same time, the need for three or more anticonvulsants was in the groups that regulate the function of mitochondria, the formation and functioning of the cytoskeleton, and the regulation of the excitability of the neuronal membrane.

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References