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Association between SCN1A gene polymorphisms and drug resistant epilepsy in pediatric patients

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ABSTRACT

Purpose: "Single Nucleotide Polymorphisms (SNPs)" could be an important explanation of drug resistance in epilepsy. The aim of this study was to investigate if genetic polymorphisms (SNPs) of the SCN1A gene could influence the response to anti – epileptic drugs (AED) and if they could predispose to a drug resistant epilepsy in pediatric patients.
Methods: We investigated SNPs in exon and intronic regions of the SCN1A gene in a sample of 120 pediatric patients, in both drug-resistant and drug-responsive patients. Association between polymorphisms and refractory epilepsy were investigated by comparing SNPs in exon and intronic regions between the two groups. The genotypes of each intronic polymorphism in the drug-resistant group was analyzed. Odds ratios and confidence intervals were calculated.
Results: None of the SNPs identified in exons of the SCN1A gene were associated with drug-resistance. In the intronic regions, a statistically significant difference was found in the prevalence of three polymorphisms was found between the two patient groups (rs6730344A/C, rs6732655A/T, rs10167228A/T). The analysis of the genotypes of each intronic polymorphism in the drug-resistant group revealed that the AA and AT genotypes for the rs1962842 polymorphism are associated with an increased risk of developing drug resistance compared to TT genotype.
Conclusion: The intronic rs6730344, rs6732655 and rs10167228 polymorphisms of the SCN1A gene are a potential risk factors for drug resistance. AA e AT genotype of the rs1962842 intronic polymorphism also emerged as a risk factor in the drug resistant group. Therefore, polymorphisms of the SCN1A gene could play a role in the response to AED in patients with drug-resistant epilepsy, with important implications for clinical practice.

1. Introduction

Epilepsy is one of the most common neurological disorders in children, with an incidence ranging from 50 to 120/100.000/year [1]. Although several AED have been developed in the course of time, it is estimated that about 20% of patients fail to respond to standard therapies and continue to experience debilitating refractory seizures [1,2]. These patients are classified as "drug-resistant epilepsy" (DRE), a clinical condition characterized by poor prognostic implications that include premature death, physical injury, psychosocial dysfunction and reduced quality of life. DRE represents a major handicap for the patient, with important repercussions on social and health costs [3]. The International League against Epilepsy task force defined DRE as the failure of adequate trials of two tolerated, appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom [3,4]. The mechanisms underlying the development of drug-resistance in epilepsy are complex and not fully understood [5]: the two well-known hypotheses for understanding the biological mechanism underlying multidrug resistance are the target and transporter hypotheses [6,7]. There is an individual variation on the optimal dose, the effectiveness and the occurrence of adverse events of an

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AED. The response of an individual patient to a specific AED is generally unpredictable [8,9]. It is influenced by many different parameters such as: the pathophysiology of the epilepsy itself; the interaction of an AED with its target(s) (pharmacodynamic effects); the pharmacokinetics of the AED, involving mechanisms of absorption, distribution, metabolism and elimination [9]. Although many factors may contribute to variability of clinical outcome in individual patients, unpredictability may partially result from genetic variation [10]. Recent developments in the pharmacogenetics of AED provide new prospects for predicting the efficacy of treatment and potential side-effects. The treatment of epilepsy appropriately offers a model for the application of pharmacogenetics in clinical practice, in view of the high prevalence of this disorder, the wide variety of individual response to drug treatment and the possibility of quantify seizure control [11]. Therefore, understanding the DRE pharmacogenetic causes is critical in order to predict drug response hence providing a basis for personalized medications.

SNPs, variations in the single-site DNA, are the most frequent forms of sequence variations in the human genome, which may affect efficacy, tolerability, safety, and duration of action of a drug and they are emerging as potential candidates of the DRE [10]. The effect of the genetic polymorphisms on the drug metabolism is significant, so we will consider how genetic variations within these processes may affect the effectiveness of AED. Following the course of an AED from its absorption in the gastrointestinal tract to drug distribution to the brain, drug action at brain targets and finally its metabolism and elimination, each process can be influenced by the presence of genetic variations that can affect transporters and target proteins, resulting in alteration of the effectiveness of the treatment [10]. The role of voltage-gated ion channels in epileptogenesis of both genetic and acquired epilepsies, as well as targets in the development of new AED, is very important [12]. Because many AED act primarily as sodium channels blockers, the SCN1A gene coding for voltage-gated sodium ion channels represents an attractive candidate for investigating the link between genetic polymorphisms and clinical response. So far, several SNPs in the sodium channel genes have been described, but only a few have been found to have a significant role in the different neurological disorders [13]. In view of the social impact that may have a poor response to treatment with AED, in terms of seizure control and adverse events, we considered the correlation between the patient's phenotype (e.g., drug response) and genotype (gene polymorphisms). Therefore, the objective of this research was to study the genetic polymorphisms that could influence the response to AED and the predisposition to develop drug resistance in patients undergoing one or more AED. The genes coding for target proteins of AED and, specifically, for voltage-gated sodium ion channels were the polimorphisms selected for this study.

2. Methods

2.1. Participants

The study included 120 epileptic pediatric patients born in Italy, attending the Neuropsychiatrics Services at the University of Bari and Salerno during the period from March 2013 and March 2014. Sixty patients were diagnosed as epileptic drug-resistant and 60 as drug-responsive. According to the criteria ILAE 2010, *drug-resistant epilepsy* was defined as "failure to achieve sustained seizure freedom, despite adequate trials of two tolerated and appropriately chosen and used AED schedules whether as monotherapies or in combination"; *drug-responsive epilepsy* was defined as "epilepsy in which the patients receiving the current AED treatment regimen has been seizure free for a minimum of three times the longest pre-intervention interseizure interval or 12 months, whichever is longer" [4].

The protocol for this study was approved by the local Ethics Committee and a written consent was obtained from patients' parents or legal guardians.

For each patient enrolled, investigators collected medical data including history of epilepsy and AED treatments received at the time of the diagnosis, reasons for any changes, dose regimens and compliance.

Inclusion criteria were: a diagnosis of epilepsy (idiopathic or cryptogenic/symptomatic), according to the International League Against Epilepsy classification [14]; treatment with at least one AED, long enough to achieve the optimal dose; drug-resistance and drug-responsiveness, according to the criteria ILAE 2010; written consent obtained from a parent or legal guardian; age between one and seventeen years.

Exclusion criteria were: epileptic patients in clinical remission, with gradual withdrawal of therapy; epileptic patients with therapy titration phase; patients with previous history of encephalitis or brain tumor.

In both drug-resistant and drug-responsive groups, we studied polymorphisms in the SCN1A gene whose mutations may be involved in mechanisms of epileptogenesis. The criteria that guided the selection of the studied SNPs are the following: we used dbSNP database of NCBI (http://www.ncbi.nlm.nih.gov/snp/) that provided the polymorphisms in the SCN1A gene with the relative allelic frequency and we chose allelic variants whose expected frequency was deemed informative. On the basis of this criteria, we investigated in exon regions SNPs rs146733308, rs35735053, rs3749029, rs112767060, rs10613159, rs12617205, rs112157737; in non-coding regions SNPs rs6730344, rs6432858, rs6732655, rs1962842, rs10167228, rs10194956 and rs11690962.

2.2. Leukocytes isolation from whole blood

Leukocytes isolation was conducted using Emagel (Piramal Healthcare, Northumberland UK) with heparin (5 U.I/ml Emagel).

Ten cc of whole peripheral blood from each patient were collected in tubes with EDTA. Equal volume of Emagel with heparin was added to blood and suspension was mixed on a rotor for 10 min. The mixture was allowed to rest to pellet red blood cells, after supernatant was collected and centrifuged at 1600 rpm for 10 min. Leukocytes were washed in 5 ml 1X PBS, centrifuged at 1600 rpm for 10 min and vortexed in 1 ml 0,2% NaCl for 1 min to remove red blood cells, then 1 ml 1.6% NaCl was immediately added. Suspension was centrifuged at 1200 rpm for 10 min, then pellet was mixed in 2 ml NaCl 0.9%. One hundred and eighty μl of this solution were collected and leukocytes were stained with 20 μl Trypan Blue solution to cell counting using Burker's chambers.

2.3. DNA extraction

DNAzol® Reagent (Life Technologies, Carlsbad CA) was employed to conduct DNA extraction. Briefly 10×10^6 leukocytes were mixed in 1 ml DNAzol® Reagent and incubated for 2–3 min at room temperature. 500 μl 100% EtOH were added to cellular suspension and tube was reversed as long as DNA was visible. The mixture was centrifuged at 10000*g* for a few seconds and supernatant was removed. The pellet was washed twice in 1 ml 75% EtOh and at finally resuspended in 100 μl 8 mM NaOH and 10 μl 0,1 M HEPES.

The DNA concentration was measured at spectrophotometer and the solution was diluted with H_2O RNasi and DNasi free (SIGMA) to obtain a final concentration 100 ng/μl.