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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.

2.4. Polymerase chain reaction (PCR)

Each polymorphic region was amplified using 100 ng DNA: 5 μl 10X PCR Buffer, 3 μl 25 mM $MgCl_2$, 2 μl 10 mM $dNTP_s$ mix, 0.5 μl AmpliTaq Gold 5U/μl (Life Technologies, Carlsbad CA) and 1 μl of specific primer (IDT Inc., Coralville, IA). Primer3 (v.0.4.0) software (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) and polymorphic sequence submitted in db SNP data base (http://www.ncbi.nlm.nih.gov/snp/) were used to draw specific primers. Table 1 shows primers and annealing temperature utilized to amplify polymorphism in SCN1A genes. Amplificates were analyzed at Gel Doc (Biorad) after electrophoresis on 2% agarose gel stained with etidium bromide.

2.5. Restriction fragment length polymorphisms (RFLP)

Ten μl of PCR products were utilized to carry out restriction fragment length polymorphism.

Endonucleases able to discriminate between wild type and polymorphic sequence were identified by NEBcutter® (http://tools.neb.com/NEBcutter2/).

The REBsites (http://tools.neb.com/REBsites/index.php) program was used instead to predict restriction fragments length. Enzymes were purchased by New England Biolabs (Ipswich, MA) and Thermo Scientific (Carlo Erba Reagents, Cornadero IT) and a digestion mixture was prepared as manufactures recommend. MslI, PsiI, SphI, ApoI, BseNI, BbsI, MspA1I, MspI, TaqI, HaeIII, *Rsa*I were employed as restriction enzymes.

2.6. Statistical analysis

Correlation between polymorphism and refractory epilepsy was investigated comparing group of drug-responsive and drug-resistant patients and calculating *P* value by *chi square* test. Within the resistant group it was analyzed whether there is a genotype that increases the risk for drug resistance. Odds ratio were calculated when $p < 0.05$.

Table 1
SCN1A polymorphisms, primers and annealing temperature. The first 7 are exonic SNPs and the last 7 are intronic SNPs.

SNPs	Primers	Ta
rs146733308G/T	FOR: 5′-CATCTTTGCCTTCTTGCTCA-3′ REV: 5′-CAAAATCAAAGGTGGGGCTA-3′	54 °C
rs35735053C/T	FOR: 5′-GTGCTTACAGACGCCACCTT-3′ REV: 5′-TGCAGTGGACATGGTCAGAT-3′	52 °C
rs3749029G/A	FOR: 5′-ttcatggcttccaatccttc-3′ REV: 5′-ttccacaattggctttgtca-3′	54 °C
rs112767060T/A	FOR: 5′-TCACATTTTTCCATCGAGCA-3′ REV: 5′-GTGGTTGTGAATGCCCTTTT-3′	54 °C
rs10613159-AAC	FOR: 5′-tgtgccatgctggtgTATTT-3′ REV: 5′-GCTGTGTGGGGAGTGGATAG-3′	52 °C
rs12617205G/A	FOR: 5′-TGGGAGTTGACAATCACTGG-3′ REV: 5′-CATTTTCCAGCTGCGAGTTT-3′	54 °C
rs112157737T/C	FOR: 5′-CATCTTTCTCTTCCCCACCA-3′ REV: 5′-TGCAAATACTTCAGCCCTTTC-3′	54 °C
rs6730344A/C	FOR: 5′-GGGGATTATACAAGGGCAAG-3′ REV: 5′-CAAAATTTGTGGAAGAAGACCA-3′	54 °C
rs6432858C/T	FOR: 5′-CAGGTGGCAAAAGCTTCATT-3′ REV: 5′-AATGTGGTGCTTCTCAAATTGT-3′	54 °C
rs6732655A/T	FOR: 5′-TCCCAAGTAGCTGGGACTACA-3′ REV: 5′-GCCTTAGAACCACCTTTATAAAA-3′	52 °C
rs1962842G/A	FOR: 5′-CATGGGCAAAATGTTGTCAG-3′ REV: 5′-CACGCCCGCCTAATTTTT-3′	54 °C
rs10167228A/T	FOR: 5′-CCAAATGGTGACACAGTGAA-3′ REV: 5′-GCCTTGATCACTTGTAGGACTTTT-3′	54 °C
rs10194956A/G	FOR: 5′-CATGGGCATGGAATAAAACA-3′ REV: 5′-CCAATCTTACACAGATTGACCA-3′	52 °C
rs11690962G/T	FOR:5′- ATCCTTGGCATCACTCTGCT-3′ REV:5′- TGATACCTTCAGTTGGGCTTTT-3′	52 °C

3. Results

A total of 120 epilepsy pediatric patients were enrolled in this study, 64 (53,3%) male and 56 (46,7%) female, 60 (50%) patients were diagnosed drug-resistant and 60 (50%) had drug-responsive epilepsy. According to the classification ILAE 1989 of epilepsy [14], in the group of drug resistant patients, 39 (65%) had localization-related epilepsy, one with idiopathic epilepsy, 27 with symptomatic, 11 with cryptogenic; 21 (35%) patients had generalized epilepsy, 8 symptomatic and 13 cryptogenic. In the group of drug responder patients, 45 (75%) had localization-related epilepsy, 35 with idiopathic epilepsy, 8 symptomatic, 2 cryptogenic; 15 (25%) patients had generalized epilepsy, 11 with idiopathic epilepsy, 3 with symptomatic and 1 cryptogenic. The average age of the patients was 11.4 years, with no significant differences between the drug-resistant (11.2 years ± 3.88 DS) versus the drug responsive patients (11.7 years ± 3.87 DS). The average age at the onset of the first seizure was 6.9 years (±4.1 DS) for the responders and 4.8 years (±3.4 DS) for the non-responders. None of the studied SNPs of the exonic region of the SCN1A gene were associated with drug-resistance. In the intronic regions of the same gene, a statistically significant difference was revealed for three of the polymorphisms studied (rs6730344A/C, rs6732655A/T, rs10167228A/T), in the genotype distribution of the two groups ($p < 0.05$). For each of the three SNP with $p < 0.05$ the odds ratio was also calculated, to evaluate the possible association between a particular genotype and drug resistance. The results were as follows:

- rs6730344A/C: the AC genotype emerged as a risk factor for drug-resistance, with O.R.=1.58 (Fig. 1);
- rs6732655A/T: the AA genotype emerged as a risk factor for drug-resistance, with O.R. =2.25 (Fig. 2);
- rs10167228A/T: the AA genotype emerged as a risk factor for drug-resistance, with O.R = 1.89 (Fig. 3).

DNA electrophoretic gels show patients genotyping for individual intronic polymorphisms (Fig. 4).

The analysis of the genotypes of each intronic polymorphism in the drug-resistant group found that rs1962842 polymorphism AA genotype (OR: 3,33) and AT genotype (OR: 2695) have a bigger risk of developing drug resistance than TT (OR: 0,348).

a) rs6730344 A*/C digested with the PsiI enzyme. Cutting with this enzyme produces two bands of 180 and 109 bp in polimorphic sequence (A*).
b)

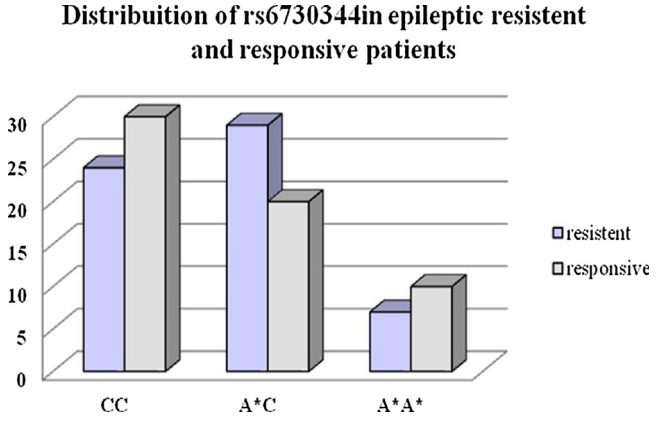

Fig 1. Polymorphic allele A is showed as *. The AC heterozygous genotype is more prevalent in the drug resistant group than in that of the drug responders.

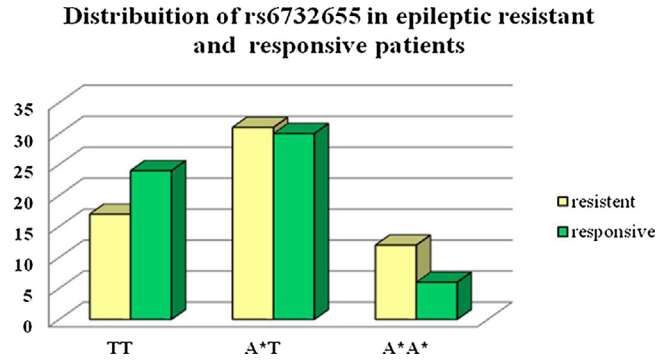

Fig. 2. Polymorphic allele A is showed as *. The AA homoozygous genotype is more prevalent in the drug resistant group than in that of the drug responders.

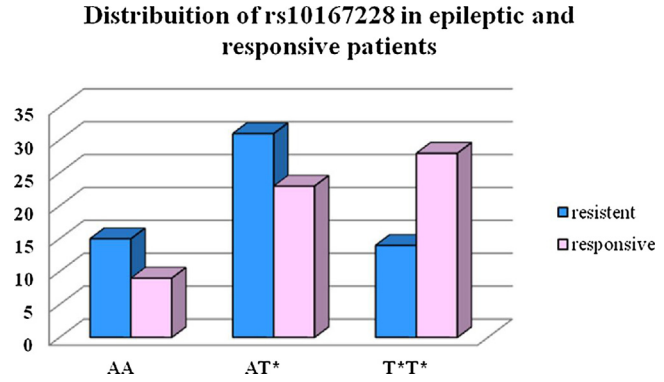

Fig. 3. Polymorphic allele T is showed as *. The AA homozygous genotype is more prevalent in the drug resistant group than in that of the drug responders.

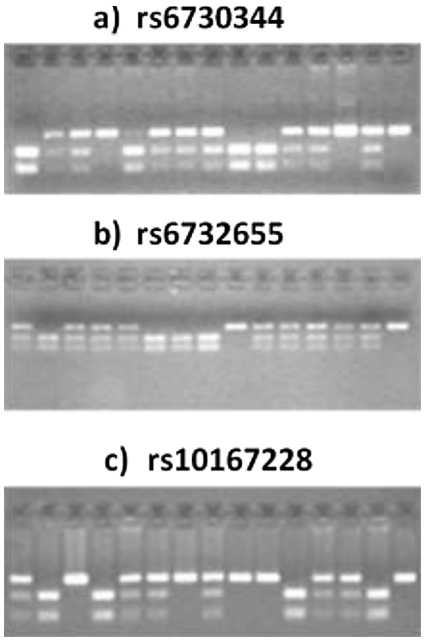

Fig. 4. DNA electrophoretic gels showing patients genotyping for individual polymorphisms.

rs6732655 A*/T genotyped with TaqI enzyme. The recognition and the respective cutting leads to formation of two bands of 95 and 155 bp in wilde type sequence (T).

c) rs10167228 A*/T. The enzyme used for this polymorphism, RsaI, produces two restriction fragments of 97 and 107 bp in polimorphic sequence (A*).

4. Discussion

Despite the widespread availability of AEDs with different mechanisms of action, more than a third of patients with epilepsy is resistant to the drugs. Twenty percent of patients in pediatric age exhibit a resistance to many AED and they are deemed to be "pharmacologically intractable". Uncontrolled crises and exposure to high doses of numerous and ineffective AED lead to significant neuropsychiatric (depression, anxiety disorder) and social impairment, lower quality of life, greater comorbidity (intellectual disability, attention and learning problems), higher risk of death [1]. Sudden unexpected death in epilepsy (SUDEP) is the most common type of death in patients with drug-resistant epilepsy. Other causes of death in patients with epilepsy may be directly related to seizures (accidental trauma, drowning, burns) or to the underlying cause of epilepsy [10]. The causes of a DRE are complex and multifactorial and this can make it difficult to obtain a statistically significant association with a SNP [15]. Because many AED act primarily as blockers of sodium channels, the gene coding for the SCN1A voltage-gated sodium channels is an interesting candidate to study the relationship between genetic polymorphisms in drug targets and clinical response [11,13,16].

The SCN1A gene, coding for the α subunit of the sodium channel voltage-dependent, is the most frequently mutated gene in different forms of epilepsy of childhood and in different types of infantile epileptic encephalopathies [17]. For the high rate of mutation in the SCN1A gene, polymorphisms were selected primarily to be found within the coding regions, to identify some causes of drug resistance in these polymorphisms.

In our study, the detection of polymorphisms (rs146733308, rs35735053, rs3749029, rs112767060, rs10613159, rs12617205, rs112157737) of the exonic regions of SCN1A gene, covering the whole study sample, did not produce statistically significant results. As for the small sample size, this result is not to be considered definitive, but rather to be confirmed enlarging the number of patients in the study.

Indeed, most of the polymorphisms present in the databases (95%) are present in the non-coding (intron, intergenic promoter or zones) which constitute the majority of the genome. Clinical trials have suggested that intronic polymorphism rs3812718G> A of SCN1A gene is involved in clinical response to treatment with AED, but the literature data in this regard are contradictory.

Tate et al. [18] reported an association between the functional polymorphism rs3812718 (c.603-91G>A) of SCN1A gene and pharmacological response, in terms of effective dosage of CBZ and PHT. Thereafter, a study involving 71 Chinese patients failed to replicate these results, although a minimal impact of the SCN1A polymorphism was not eliminated [19]. Two other replication studies – the first carried out on 228 Japanese epileptic patients [20] and the second on 369 Australian epileptic patients [21] – have failed to identify an association between the polymorphism and doses of sodium blockers drugs, although the Japanese study described an association between genotype AA of c.603-91 polymorphism and unresponsiveness to CBZ [13,20]. These different data could be explained by many factors, including size and heterogeneity of the sample, selection bias and other confounding factors. Furthermore, we must consider the limited support of a single SNP: in fact, as the genetic component of the drug response is usually polygenic [10], the impact of a single gene could be influenced by other genes and by environmental factors [13].

Recently, Yip et al. [16] have studied nine SNPs within SCN1A in 519 Caucasian epileptic patients with known response status for sodium channel blocking AED: firstly, the only association observed was for rs10188577; after correction for potential confounding factors, the association for rs10188577 was only marginally significant (P = 0.049). Therefore, it seems unlikely that rs10188577 could be a major determinant of response to AED [16]. We investigated polymorphisms rs6730344, rs6432858, rs6732655, rs1962842, rs10167228, rs10194956 and rs11690962 within the intronic regions of the SCN1A gene, in both drug-responsive and drug-resistant groups. As these polymorphisms are located in intronic regions, their presence could interfere with the mechanisms of alternative splicing and therefore alter the conformation of the functional domains of the Na^+ channel; moreover, they could result in unstable primary transcript and therefore in decreased synthesis of the Na^+ channel. Our study revealed a statistically significant difference ($P < 0.05$) on the distribution of genotypes between the two groups of epileptic patients (drug resistant and drug sensitive) for three of these polymorphisms (rs6730344, rs6732655 and rs10167228), allowing to assign to these polymorphisms a role in the response to antiepileptic drugs. In particular, assessing a possible association between a specific genotype and phenotype drug resistance by odds ratio, we found that the genotype rs6730344 A/C (O.R. = 1.58), rs6732655 A/T (O.R. = 2.25) and rs10167228 A/T (O.R. = 1.89) appear to be related to the drug resistance phenotype, as they have a prevalent genotype distribution in the group of drug-resistant patients. We also investigated whether within the group of patients with drug-resistant epilepsy there was a significant genotype for drug resistance risk. Rs1962842 polymorphism AA genotype (OR: 3,33) and AT genotype (OR: 2695) have an increased risk of developing drug resistance than TT (OR: 0,348) among the seven analyzed intronic polymorphisms (rs6730344, rs6432858, rs6732655, rs1962842, rs10167228, rs10194956 and rs11690962).

How could polymorphisms located in non-coding regions affect the response to drugs? The presence of significant polymorphisms located in non-coding regions has stimulated us to further investigate the significance of this result. We used Conserved domain software (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cg) to determine the domains present in the sequence of the gene SCN1A. Then, each polymorphism in our study was put in relation to the functional domains of the protein. Rs10167228 is positioned between two exons that encode for a domain whose function has not been characterized, DUF34511. This domain has been found only in eukaryotes and it is associated to other functional domains as pfam06512 (Na_trans_assoc) and pfam00520 (Ion_trans).

rs6730344 (p = 0.0462 rs6730344 AC o.r = 1.58) is located between a region that encode for pfam06512. This domain, also known as Na_trans_assoc, includes a region found exclusively in eukaryotic sodium channels or their subunits, many of which are voltage-gated. Members very often also contain between one and four copies of pfam00520 (Ion_trans) and, less often, one copy of pfam00612 (IQ calmodulin-binding motif).

rs6732655 (p = 0.017 rs6732655 AA o.r. = 2.25) is surrounded by exons coding for the domain pfam00520 (Ion_trans). This family is characterized by 6 transmembrane helices in which the last two helices flank a loop which determines ion selectivity and in sodium channels the domain is repeated four times. However, rs6732655 in isoforms 1, 2 and 3 (respectively NM_001165963, NM_006920 and NM_001165964) is located between two exons that in addition to code for pfam00520 also encode for cl21557 domain (Yip1). The Yip1 integral membrane domain contains four transmembrane alpha helices characterized by the motifs DLYGP and GY.

It has been widely acknowledged that introns are an integral part of the regulation of gene expression. Non-coding RNAs within introns are commonly produced through the post-splicing process and are specific signals of gene transcription events, impacting many other genes and modulating their expression. An association between human introns and ncRNAs has a pronounced synergistic effect with important implications for fine-tuning gene expression patterns across the entire genome [22].

Therefore, although introns are non-coding regions, the presence of polymorphisms in these sequences may result in alterations in the encoded protein, because intronic polymorphic sequences flanking exons can result in destruction of existing splicing sites or in the creation of new ones [23]. Moreover, the presence of intronic polymorphisms could interfere with the formation of the spliceosome [24,25].

5. Conclusion

Considering the psychosocial impact of drug-resistant epilepsy, the knowledge of the mechanisms of drug resistance becomes important in the development of AED and clinical practice. Pharmacogenomics constitute an emerging field with high potential to influence our decisions for the treatment of epilepsy in the future. Literature data report associations between specific genetic polymorphisms and drug resistance; although these results may not always be replicated in subsequent studies, however, the study of polymorphisms of genes involved in the metabolism of AED is a very attractive research line.

Finally, our study showed a significant association between rs6730344, rs6732655 and rs10167228 polymorphisms in the intronic regions of the SCN1A gene and refractory epilepsy, thus emerging as risk factors for drug resistance. SCN1A gene polymorphisms may play a role in the response to AED in patients with drug-resistant epilepsy, with important implications for clinical practice, which has the highest goal of a tailored therapy. These pharmacogenetic data could assist clinicians in predicting patient drug response phenotype and allow for molecular-based design of genotype specific therapeutics with greater tolerability and efficacy. This could be the basis of a personalized epilepsy treatment aiming at improving patient quality of life. Further studies are needed to confirm these results and complete our journey through the pharmacogenetic causes of drug resistant epilepsy.

Multicenter studies will be necessary to reach the goal of a broader understanding of pharmacogenomic mechanisms in the epilepsies. Moreover, identification of polymorphisms of candidate genes, which may influence AED response and resistance, could lead to new knowledge of the molecular mechanisms underlying drug-resistant epilepsy and the development of new and more effective drugs.

Conflicts of interest

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References

[1] Wirrell EC. Predicting pharmacoresistance in pediatric epilepsy. Epilepsia 2013;54(Suppl. S2):19–22.
[2] Pohlmann-Eden B, Weaver DF. The puzzle(s) of pharmacoresistant epilepsy. Epilepsia 2013;54(Suppl. 2):1–4.
[3] Téllez-Zenteno JF, Hernàndez-Ronquillo L, Buckley S, Zahagun R, Rizvi S. A validation of the new definition of drug-resistant epilepsy by the International League Against Epilepsy. Epilepsia 2014;55(6):829–34.

[4] Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on therapeutic strategies. Epilepsia 2010;51:1069–77.
[5] Sánchez MB, Herranz JL, Leno C, Arteaga R, Oterino A, Valdizán EM, et al. Genetic factors associated with drug-resistance of epilepsy: relevance of stratification by patient age and aetiology of epilepsy. Seizure 2010;19:93–101.
[6] Rogawski MA, Johnson MR. Intrinsic severity as a determinant of anti-epileptogenic drug refractoriness. Epilepsy Curr. 2008;8:127–30.
[7] Kasperaviciute D, Sisodiya SM. Epilepsy pharmacogenetics. Pharmacogenomics 2009;10:817–36.
[8] Klamer S, Singh A, Gil-Nagel A, Kramer G, French J, Lerche H. Current recommendations, guidelines, and expert views of practical anticonvulsant therapy. In: Lerche H, Potschka H, editors. Therapeutic targets and perspectives in the pharmacological treatment of epilepsy. Germany: UNI-MED Verlag AG; 2013. p. 26–36.
[9] Weber YG, Nies AT, Schwab M, Lerche H. Genetic biomarkers in epilepsy. Neurotherapeutics 2014;11:324–33.
[10] Löscher W, Klotz U, Zimprich F, Schmidt D. The clinical impact of pharmacogenetics on the treatment of epilepsy. Epilepsia 2009;50:1–23.
[11] Szoeke CE, Newton M, Wood JM, Goldstein D, Berkovic SF, OBrien TJ, et al. Update on pharmacogenetics in epilepsy: a brief review. Lancet Neurol 2006;5:189–96.
[12] Kumari R, Lakhan R, Garg RK, Kalita J, Misra UK, Mittal B. Pharmacogenomic association study on the role of drug metabolizing, drug transporters and drug target gene polymorphisms in drug-resistant epilepsy in a north Indian population. Indian J Hum Genet 2011;17(Suppl. 1):S32–40.
[13] Manna I, Gambardella A, Bianchi A, Striano P, Tozzi R, Aguglia U, et al. A functional polymorphism in the SCN1A gene does not influence antiepileptic drug responsiveness in Italian patients with focal epilepsy. Epilepsia 2011;52(5):e40–4.
[14] Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 1989;30:389–99.
[15] Depondt C. The potential of pharmacogenetics in the treatment of epilepsy. Eur J Pediatr Neurol 2006;10:57–65.
[16] Yip TS, O'Doherty C, Tan NC, Dibbens LM, Suppiah V. SCN1A variations and response to multiple antiepileptic drugs. Pharmacogenomics J 2014;14(4):385–9.
[17] Zucca C, Redaelli F, Epifanio R, Zanotta N, Romeo A, Lodi M, et al. Cryptogenic epileptic syndromes related to SCN1A. Twelve novel mutations identified. Arch Neurol 2008;65(4):489–94, doi:http://dx.doi.org/10.1001/archneur.65.4.489.
[18] Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, et al. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. Proc Natl Acad Sci U S A 2005;102:5507–12.
[19] Tate SK, Singh R, Hung CC, Tai JJ, Depondt C, Cavalleri GL, et al. A common polymorphism in the SCN1A gene associates with phenytoin serum levels at maintenance dose. Pharmacogenet Genomics 2006;16:721–6.
[20] Abe T, Seo T, Ishitsu T, Nakagawa T, Hori M, Nakagawa K. Association between SCN1A polymorphism and carbamazepine-resistant epilepsy. Br J Clin Pharmacol 2008;66:304–7.
[21] Zimprich F, Stogmann E, Bonelli S, Baumgartner C, Mueller JC, Meitinger T, et al. A functional polymorphism in the SCN1A gene is not associated with carbamazepine dosages in Austrian patients with epilepsy. Epilepsia 2008;49:1108–9.
[22] Rearick D, Prakash A, McSweeny A, Shepard SS, Fedorova L, Fedorov A. Critical association of ncRNA with introns. Nucleic Acids Res 2011;39(6):2357–66.
[23] Markovic D, Challiss RA. Alternative splicing of G protein –coupled receptors: physiology and pathophysiology. Cell Mol Life Sci 2009;66(20):3337–52.
[24] Evsyukova I, Bradrick SS, Gregory SG, Garcia-Blanco MA. Cleavage and polyadenylation specificity factor 1 (PSF 1) regulates alternative splicing of interleukin 7 receptor (IL7R) exon 6. RNA 2013;19(1):103–15.
[25] Lee Y, Rio DC. Mechanisms and regulation of alternative pre-mRNA splicing. Ann Rev Biochem 2015;84:291–323.