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No association between common variations in the human alpha 2 subunit gene (ATP1A2) of the sodium–potassium-transporting ATPase and idiopathic generalized epilepsy

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Abstract

Quantitative trait loci studies in inbred mice have identified a locus on chromosome 1 (*Szs1*) of fundamental importance to seizure susceptibility. High-ranking candidate genes in this susceptibility region include KCNJ9, KCNJ10 and ATP1A2. We performed a systematic mutation scan of the coding region of the human ATP1A2 gene and performed a case–control association study with seven common markers. Genotypes were assessed in 152 idiopathic generalized epilepsy (IGE) patients of German ancestry and 111 healthy German controls for all seven polymorphisms. No significant differences were found in genotype or allele frequencies for any of the variations between the IGE patients and controls. No haplotypes were associated with IGE when compared to controls. Linkage disequilibrium was demonstrated throughout the gene. Results suggest that the polymorphisms we studied in the ATP1A2 gene do not represent major susceptibility factors for common forms of IGE.

Keywords: Na–K-ATPase; Seizure; DNA polymorphism; Genetics; Association

Hereditary factors play a predominant role in the etiology of idiopathic generalized epilepsy (IGE), which accounts for 30% of all epilepsies [9,15,34]. However, genetic causes of the common IGE syndromes are difficult to identify due to the complex mode of inheritance and genetic heterogeneity [22,28,38].

In our laboratory we use a polygenic mouse model to identify genes which influence seizure susceptibility. Quantitative trait loci (QTL) mapping and congenic animal studies with inbred mice, which display focal and generalized seizures depending on the used seizure induction method [10,12,13], have led to the identification of a critical region on distal mouse chromosome 1 (*Szs1*) which harbors a gene or genes that contribute to both generalized and focal seizure susceptibility [10–13]. High priority candidate genes in this seizure susceptibility region and the homologous human region (1q21–23) include KCNJ9, KCNJ10 and ATP1A2 [4,11].

Several lines of evidence make ATP1A2 a plausible candidate for an IGE susceptibility gene. The sodium–potassium pump is involved in regulation of ion homeostasis across the cell membrane and several rare forms of epilepsy are caused by mutations in genes controlling ion homeostasis [38]. Recently, mutations in the ATP1A2 gene were shown to cause familial hemiplegic migraine (FHM) [1,7,21,36,39]. Interestingly, members of two families had benign familial infantile convulsions [40] or generalized seizures [36] in addition to hemiplegic migraine episodes [19]. Further studies in patients with epilepsy and experimental animal

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models suggest a relationship between Na–K-pump activity and seizures [18,20,23,24,27,30,33]. Mutations in the ATP1A2 gene may cause seizures given the fundamental role of the ATPase enzyme in regulating neuronal membrane potential and hence neuronal firing [17,25,26,35].

In this study we performed a systematic mutation scan of the ATP1A2 coding region and tested the hypothesis that common variations in the ATP1A2 gene confer susceptibility to IGE.

IGE patients were collected at the Department of Neurology, Charité University Medicine Berlin, Germany. The study protocol was approved by the Institutional Ethics Committee. Written informed consent was obtained from all participants who were unrelated individuals of German descent. The study sample included 152 IGE patients, comprising 37 probands with childhood absence epilepsy (CAE), 32 probands with juvenile absence epilepsy (JAE), and 83 probands with juvenile myoclonic epilepsy (JME). The diagnostic classification of IGE syndromes was performed according to the revised version of the 'Classification of Epilepsies and Epileptic Syndromes' of the International League Against Epilepsy [6]. Diagnostic criteria for JME were: (a) characteristic repetitive bilateral myoclonic jerks in shoulders and arms without loss of consciousness, occurring after awakening; (b) age-of-onset of typical myoclonic seizures between 8 and 25 years. Diagnostic criteria for idiopathic absence epilepsy (CAE or JAE) were: (a) begin with typical absence seizures (brief spells of generalized onset with loss of consciousness); (b) age at onset of typical absence seizures between 3 and 20 years. Common inclusion criteria were: (a) EEG with normal background activity and paroxysmal GSW–EEG discharges; (b) normal intellectual and neurological status beside seizures. General exclusion criteria included: (a) evidence for structural lesions, metabolic or degenerative diseases of the brain; (b) atonic/astatic or tonic seizures; (c) complex partial seizures; (d) epilepsy with myoclonic absences; (e) exclusively stimulus induced seizures.

The population controls included 111 healthy German individuals with no history of seizures or chronic neurological disease.

Peripheral blood samples were obtained and genomic DNA was extracted from peripheral leukocytes by standard procedures.

The ATP1A2 gene consists of 23 exons and spans about 26 kb (NCBI Access # J05096). Review of the public database, the Celera database and the literature revealed one synonymous single nucleotide polymorphism (SNP) in the coding region of the ATP1A2 gene and at least 91 non-coding SNPs. Several missense mutations have been reported in patients with FHM [1,7,21,36,39].

All 23 exons and intron–exon boundaries were systematically screened for mutations in 30 individuals with epilepsy and 30 controls using single strand conformation polymorphism (SSCP) analysis as described [2]. Details of intron–exon structure and primer sequences are available upon request. PCR conditions, genotyping and primer pairs for the intron 1 ins-6704/TTCC variation were described previously [3]. All SNPs were confirmed by subcloning and sequencing PCR amplicons from at least three separate PCR reactions from different individuals.

The intron 1 ins-6704/TTCC and intron 22 variations were genotyped using SSCP analysis. The exon 9 SNP introduced a *Dde*I restriction site allowing genotyping by standard agarose gel electrophoresis and ethidium bromide staining of the restriction fragment length polymorphism (RFLP).

Additional SNPs for genotyping were chosen based on availability of *Applied Biosystems* assays-on-demand, location in the gene and allele frequencies. Genotyping of four additional intronic SNPs across the ATP1A2 gene (Fig. 1) was performed using the *Applied Biosystems* "Assays-on-demand" SNP genotyping assay as per manufacturers protocol (C_7479749_10/rs1016732, C_1843218_10/rs1407130, C_1843220_10/rs6686067, C_15867718_10/rs2070702).

Genotypes and allele frequencies were compared between groups using chi-square contingency analysis. A two-tailed type I error rate of 5% was chosen for the analysis. No correction for multiple testing was made based on the rational that this is an explorative approach to a genetically complex disorder in which a phenotype–genotype relationship has not been established [32]. Our sample size had reasonable power to detect a disease association at a *P*-value less than or equal to 0.05, assuming a genetic effect of 2.0 (Table 1). Power analysis was performed using the *Quanto* program [16]. Linkage disequilibrium (LD) was calculated using the *2ld* program [41]. Haplotype frequencies were estimated using the *EH* program [29] and *Cocaphase* program [8].

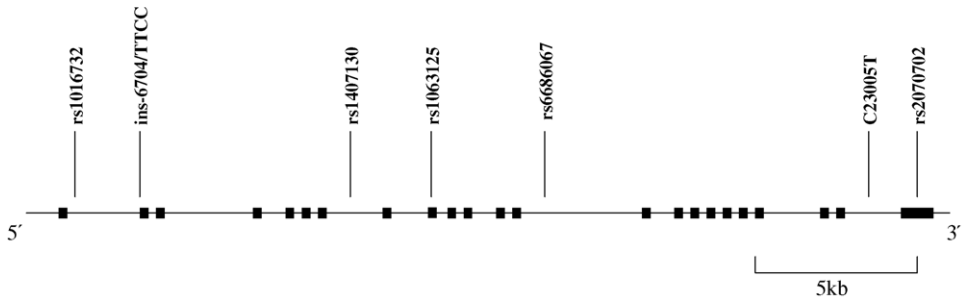

Fig. 1. Variations in the ATP1A2 gene.