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Polymorphisms in the GluR2 gene are not associated with amyotrophic lateral sclerosis

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Abstract

Excitotoxicity is thought to play a pathogenic role in amyotrophic lateral sclerosis (ALS). Excitotoxic motor neuron death is mediated through the Ca²⁺-permeable AMPA-type of glutamate receptors and Ca²⁺ permeability is determined by the GluR2 subunit. We investigated whether polymorphisms or mutations in the GluR2 gene (*GRIA2*) predispose patients to ALS. Upon sequencing 24 patients and 24 controls no non-synonymous coding variants were observed but 24 polymorphisms were identified, 9 of which were novel. In a screening set of 310 Belgian ALS cases and 794 healthy controls and a replication set of 3,157 cases and 5,397 controls from 6 additional populations no association with susceptibility, age at onset or disease duration was observed. We conclude that polymorphisms in the GluR2 gene (*GRIA2*) are not a major contributory factor in the pathogenesis of ALS.

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Keywords

Amyotrophic lateral sclerosis; excitotoxicity; GluR2; motor neuron

1. Introduction

Glutamate-induced cell death is thought to play a pathogenic role in amyotrophic lateral sclerosis. Excitotoxic motor neuron death is mediated by extensive Ca^{2+} entry into the cell through Ca^{2+} -permeable AMPA receptors (Van Den Bosch et al., 2006). The Ca^{2+} permeability of AMPA receptors is determined by the presence or absence of the GluR2 subunit in the receptor complex. We have previously shown that lowering GluR2 aggravates mutant SOD1-induced motor neuron degeneration in mice (Van Damme et al., 2005), while Tateno et al. demonstrated that overexpression of GluR2 slowed down the motor neuron loss in this ALS model (Tateno et al., 2004). The question arises whether genetically determined differences in GluR2 expression could make humans more susceptible to diseases in which excitotoxicity is pathogenically involved. We therefore investigated whether polymorphisms in the GluR2 gene (*GRIA2*) are a risk factor for the development of ALS or a modifier of the disease phenotype.

2. Methods

The first sets of sporadic ALS patients were recruited from the university hospitals of Leuven and Antwerp. The control population consisted of healthy individuals from the same geographical area. The replication populations of in total 3,157 cases and 5,397 controls are described in references: Landers et al., 2009; Schymick et al., 2007; van Es et al., 2007; van Es et al., 2008 and van Es et al., 2009. Additional control data from the UK were obtained from 1,428 individuals from the British 1958 Birth Cohort DNA Collection (data deposited by the Wellcome Trust Sanger Institute and published online [<http://www.b58cgene.sgu.ac.uk/>]). SNP detection and genotyping was performed as described in the supplementary material.

3. Results

Because of the importance of AMPA receptor mediated excitotoxicity and more particularly the GluR2 subunit in ALS (Van Den Bosch et al., 2006), our aim was to clarify whether SNPs in the GluR2 gene could be a potential genetic risk factor for sporadic ALS. In order to investigate this hypothesis, we first identified the SNPs present in the *GRIA2* gene. Sequencing all exons, including exon-intron boundaries and the 5' and 3' untranslated region of the *GRIA2* gene in a subset of 24 ALS patients and 24 controls revealed 24 SNPs, of which 9 are unknown in the public SNP database (Ensembl release 54, July 2008) (Supplementary Table 1). Linkage disequilibrium analysis revealed 5 haplotype-tagging (ht) SNPs that capture 80% of the overall haplotype diversity. The 5 htSNPs were genotyped in 310 sporadic ALS cases and 794 controls. The frequency of rs6826285*T was lower in cases than controls ($P=0.04$, Supplementary Table 2). Adjusting for age and gender did not change these observations significantly. None of the SNPs was associated with age at onset ($P>0.37$). Genotype of none of the htSNPs was associated with survival in the Belgian study population while adjusting for age at onset and site of onset ($P>0.25$). Genotyping of SNP rs6826285 requires direct sequencing but the SNP is highly correlated (concordance 122/124 alleles, $D'=0.92$, $r^2=0.84$) with rs10025251, which can be genotyped with high-throughput genotyping technologies. Hence we sought to corroborate the trend observed in the Belgian study population for rs6826285 by investigating rs10025251 in other available datasets from France, the Netherlands, Poland, Sweden, the UK and the US. The total study population

consists of 3,467 cases and 6,191 controls. Overall genotyping success rate is >98%. Power is 99% to observe $P < 0.05$ and 76% to observe $P < 10^{-3}$ for an odds ratio of 1.2 in the replication dataset, taking into account D' . No heterogeneity between populations was observed (Breslow-Day $P = 0.36$). A meta-analysis using the Cochran-Mantel-Haenszel test stratified over country of origin resulted in $P = 0.40$ for all populations and $P = 0.88$ for the replication dataset (Figure 1).

4. Discussion

We found no evidence for an association of GluR2 SNPs with ALS. However, this does not exclude a differential GluR2 regulation between ALS patients and control individuals as there are multiple other mechanisms described that regulate GluR2-subunit-containing AMPARs (Isaac et al., 2007). GluR2 mRNA is regulated by editing, splicing and mRNA transport to dendrites and its local translation (Isaac et al., 2007; Ju et al., 2004). In the context of ALS, this becomes particularly interesting as mutations in TDP-43 and FUS/TLS, which are RNA binding molecules, were recently discovered as genetic causes of the disease (Lagier-Tourenne and Cleveland, 2009). Moreover, assembly of the receptor complex and incorporation of the GluR2 lacking AMPA receptors into the synapse are also tightly regulated processes that could be disturbed in ALS or could predispose patients to develop the disease (Isaac et al., 2007). In addition (growth) factors secreted by surrounding cells, which are also thought to play a dominant role in the pathogenesis, could also influence the expression level of the GluR2 subunit. We recently showed that differences in astrocytic factors modulate the GluR2 expression in motor neurons and so the susceptibility of motor neurons to excitotoxicity (Van Damme et al., 2007). Furthermore the vascular endothelial growth factor, which is also secreted by astrocytes, is capable of inducing GluR2 expression levels (Bogaert et al., 2009). In conclusion, we observed a lack of association in the GluR2 subunit in SALS which doesn't exclude the possibility that GluR2 is differentially regulated in ALS patients and controls.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Meta-analysis in a study population of 3,467 cases and 6,191 controls. The abbreviations stand for Belgium (BEL), France (FRA), the Netherlands (NL), Poland (PL), Sweden (SWE), the United Kingdom (UK) and the United States (US).