Supplementary Material for Website

No association between DNA repair gene *XRCC1* and amyotrophic lateral sclerosis

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Abstract

Reduced DNA repair capacity may play a role in amyotrophic lateral sclerosis (ALS) etiology. We examined the association between the risk of ALS and single nucleotide polymorphisms (SNPs) in the gene x-ray repair complementing defective repair in Chinese hamster cells 1 (*XRCC1*) utilizing data from a case-control study in New England and two genome-wide association studies (the Study of Irish Amyotrophic Lateral Sclerosis and the NINDS genome-wide study in Amyotrophic Lateral Sclerosis and Neurologically Normal Controls). Our results did not show any differences in the frequency of *XRCC1* gene polymorphisms between ALS patients and controls free of any neurological disease.

Introduction

Abnormal capacity to repair DNA damage has been suggested to play a role in the pathogenesis of amyotrophic lateral sclerosis (ALS) (Bradley and Krasin, 1982). Neurons, especially motor neurons, are sensitive to reactive oxygen species (ROS) and to DNA damage such as apurinic/apyrimidinic sites and single-strand and double-strand breaks induced by ROS (Martin and Liu, 2002). Oxidative DNA damage has been detected in the spinal cord tissue of ALS patients (Fitzmaurice et al., 1996). Defects in the base-excision repair (BER) pathway, a major pathway counteracting the effects of ROS-induced DNA damage, may thus play a role in ALS development. Three key genes of the BER pathway, 8-oxoguanine DNA glycosylase (*OGG1*), APEX nuclease (multifunctional DNA repair enzyme) 1 (*APEX1*), and x-ray repair complementing defective repair in Chinese hamster cells 1 (*XRCC1*) were suggested to influence the development of chronic diseases involving postmitotic neurons (Martin, 2008). The associations between ALS risk and *OGG1* or *APEX1* have been investigated previously although the results are not conclusive (Coppede et al., 2008; Coppede et al., 2007; Hayward et al., 1999; Kikuchi et al., 2002; Kisby et al., 1997; Olkowski, 1998). To our knowledge there are no data reported to date on the role of *XRCC1* on ALS. In the present study, we assessed the associations between 14 single nucleotide polymorphisms (SNPs) in *XRCC1* and ALS risk.

Materials and Methods

We first used data from a case-control study conducted in New England between 1993 and 1996 (Kamel et al., 2002). Cases were recruited from two major referral centers for ALS, with diagnosis confirmed by a board-certified neurologist. Population controls were identified through random telephone screening. Controls with no physician-diagnosed

neurodegenerative disease were frequency matched to cases on gender, age (30-55, 56-65, and 66-80 years), and region within New England (Boston metropolitan area, eastern Massachusetts, and rest of New England). The present analysis includes 108 cases and 39 controls with blood samples, among whom 95% were white and not Hispanic.

We selected for genotyping three nonsynonymous SNPs in *XRCC1* (rs25487 G/A Arg399Gln, rs1799782 C/T Arg194Trp, and rs25489 G/A Arg280His) that have been frequently studied, one synonymous SNP (rs915927 A/G Pro206Pro), and one intronic SNP (rs939461 A/C). These five SNPs can distinguish all common haplotypes (frequency cutoff = 5%) reconstructed using software PHASE 2.0 (Stephens et al, 2001) on dbSNP genotype data of the European population for all common SNPs. The SNPs were not in strong LD; the highest pairwise correlation (r^2) among the five SNPs was 0.15. DNA was extracted from frozen blood samples using Gentra PUREGENE reagents (Gentra Systems, Minneapolis, MN) and frozen until used. SNPs rs939461, rs915927, and rs25489 were genotyped using the MassARRAYTM system (Sequenom, San Diego CA), and SNPs rs25487 and rs1799782 were genotyped using the MasscodeTM system (Qiagen Genomics Inc, Bothell WA) by BioServe Biotechnologies, Ltd (Laurel MD). Use of these two methods ensured over 95% genotype call rates for all SNPs.

We checked for consistency of genotypic and allelic frequencies observed among controls with those expected assuming Hardy-Weinberg equilibrium using an exact test (proc Allele, SAS, Cary, North Carolina) and no deviation was detected ($p > 0.26$ for each locus).

Exact logistic regression methods were applied to assess the odds ratios (ORs) of ALS among carriers of a specific genotype compared to carriers of the most common homozygotic genotypes for each SNP. Analyses were performed using LogXact version 8 (Cytel Software Corp, Cambridge, MA). All study participants provided written informed consent. The study

proposal was approved by the Institutional Review Boards of the National Institute of Environmental Health Sciences, Tufts-New England Medical Center, and Brigham and Women's Hospital.

Genome-Wide Association Study (GWAS) data

The Study of Irish Amyotrophic Lateral Sclerosis (221 cases and 211 controls with Irish Caucasian ethnicity for at least three generations) (Cronin et al., 2008; dbGAP accession number phs000145.v2.p2) and the NINDS Genome-wide Genotyping in Amyotrophic Lateral Sclerosis and Neurologically Normal Controls Study (276 cases and 271 controls that were white and non-Hispanic individuals) (Schymick et al., 2007; dbGAP accession number phs000101.v2.p1) have been described in detail previously. We utilized the publically available data from these two studies to examine all reported SNPs in the *XRCC1* gene. A total of 10 relevant SNPs were genotyped in both GWAS and were included in our analysis. We used logistic regression models separately for each study to derive the ORs and 95% CIs for each SNP.

Results and Discussion

In the New England ALS study, a lower risk of ALS was noted among carriers of the variant genotypes for rs25487 and rs939461 (Supplemental material Table 1); these two SNPs were not in strong LD with each other among the controls ($r^2 = 0.17$). The associations between ALS risk and other SNPs were not clear. No association between any of the 10 SNPs and ALS risk was noted in the Irish GWAS. An association between variant genotype of rs939461 and a higher risk of ALS was noted in the NINDS GWAS, but the estimate was of only borderline statistical significance (Supplemental material Table 2).

DNA damage, possibly due to excessive ROS coupled with defective DNA repair, may be an upstream mechanism for neurodegeneration in ALS (Shaikh and Martin, 2002). Key genes in the BER pathway are believed to be the major pathway for repairing DNA bases with oxidative damage (Hegde et al., 2008). Missense mutations in *APEX1* were associated with ALS risk in both sporadic and familial ALS in one study (Olkowski, 1998), but not in other studies (Coppede et al., 2008; Hayward et al., 1999). An association between the Ser326Cys polymorphism in *OGG1* and a higher risk of ALS was reported earlier (Coppede et al., 2007). To our knowledge, no study has reported a relationship between ALS and *XRCC1*, a gene considered as an essential element in repair of damaged bases and single-strand breaks (Ladiges, 2006).

The three most studied polymorphisms of *XRCC1* in cancer are rs25487 (Arg399Gln), rs25489 (Arg280His), and rs1799782 (Arg194Trp). We noted a hint of an association between rs939461 and a lower risk of ALS in the New England ALS Study, but given the small sample size no sound conclusion could be drawn. The different result observed in the NINDS GWAS as well as the null finding in the Irish GWAS further suggests that the observed associations may be due

to chance. In summary, single marker analysis did not reveal evidence of a significant association of *XRCC1* variants with ALS risk in this analysis.

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