Cigarettes and Amyotrophic Lateral Sclerosis: Only Smoke or Also Fire?

The search for nongenetic risk factors for amyotrophic lateral sclerosis (ALS) has progressed slowly over the past decades. Although numerous hypotheses have been proposed, outside of age and male gender, no risk factor has emerged as a consistent and accepted predictor of risk. The lack of rigorous longitudinal studies of ALS has probably been one of the causes. Although classical case-control studies have played a critical role in the history of epidemiology, and still provide a useful tool in many settings, the elucidation of the role of behavioral and environmental factors in the etiology of chronic diseases will ultimately require the prospective observation of large cohorts. The report by Gallo et al in this issue of *Annals of Neurology*[1] is the third cohort study with prospectively collected data to address the question of smoking and risk of ALS.

Over the past decade, smoking has gone from being presumed unrelated to ALS to being a leading candidate as an environmental predictor of ALS. The results of early case-control studies suggested no association with ALS, but they were fraught with methodological problems. Some were more obvious, such as small sample sizes and unreliable data on cigarette smoking behavior; others were somewhat more subtle, such as choosing friends or spouses of cases as controls. Because the smoking habits of friends and spouses tend to be similar, this choice of controls would mask any true association between smoking and ALS. As methodological savvy improved, so did the studies, and two more recent case-control studies with improved methodology each suggested an increased risk of ALS among smokers,[2,3] as did one that used acquaintances as controls, which might have been expected to bias any association towards the null.[4] Although these are vast improvements over their predecessors, issues of recall bias and control selection can play out in unpredictable and sometimes imperceptible ways in case-control studies. The prospective cohort design is mostly free from recall bias and, with suitable follow-up, is also less prone to the selection biases that can creep into case-control studies. But in the context of ALS this study design had been rarely implemented, because the follow-up of a very large population, which is prohibitively expensive, is necessary to achieve sufficient statistical power. This obstacle is now being circumvented by taking advantage of large cohorts of individuals who have been recruited and followed to investigate risk factors for other chronic diseases.

Gallo and colleagues take advantage of the European Prospective Investigation into Cancer and Nutrition cohort recruited from the general population at several sites in Europe and assembled to address questions of nutrition and cancer. This large cohort of 517,890 participants provided 4,591,325 person-years of follow-up, over which time 118 deaths from ALS were identified. They found current smokers at baseline to be almost twice as likely to have ALS listed as a cause of death in their death certificates than never smokers (hazard ratio: 1.89; 95% confidence interval: 1.14–3.14). Further, the risk of death from ALS increased with years of smoking (p for trend = 0.002), and decreased with years since quitting. Although these results unambiguously support an adverse effect of smoking on ALS incidence or mortality, they should be interpreted in the context of partly contradictory findings in two previous longitudinal investigations.

In a longitudinal study larger than that of Gallo et al that comprised nearly 1 million people and 621 deaths (330 of which were men), we reported a few years ago an excess risk of death from ALS among female smokers, but not among male smokers.[5] More recently, Fang et al reported a lack of association between smoking and ALS risk in a study comprising over 280,000 male construction workers in Sweden, 160 of whom were diagnosed with ALS.[6] Although Gallo et al reported that a positive association between smoking and ALS was observed in both men and women, there were only 40 ALS deaths among men, and the excess ALS risk among male smokers alone was not significant. Thus, although the results of this study strengthen the evidence implicating smoking as a risk factor for ALS, there is still a substantial degree of uncertainty, and the possibility of differences by gender cannot yet be ruled out. Further, it will be important to compare in clinical studies the rates of clinical progression and the survival curves of smokers and nonsmokers with ALS, to determine whether the higher death rates from ALS among smokers could be in part explained by a shorter disease duration.

These uncertainties notwithstanding, the possibility that cigarette smoking increases the risk of ALS is intriguing from a mechanistic perspective. Cigarette smoke inhibits paraoxonase (PON),[7] an enzyme that contributes to reducing the damage from oxidative stress. Slow metabolizer polymorphisms of the PON genes have been associated in some[8,9] but not all[10] studies with an increased risk of ALS. Cigarette smoke also inhibits the vascular endothelial growth factor (VEGF) signaling pathway.[11] Although VEGF genetic polymorphism studies in humans have been equivocal regarding an association with ALS, in vitro and transgenic animal studies provide strong evidence for a protective role for VEGF on motor neuron survival.[12] Intriguingly, a major component of cigarette smoke—and one suggested to be responsible for the inhibition

of PON activity[7]—is formaldehyde,[13] exposure to which we recently found to be associated with an increased risk of ALS.[14] Conclusions will have to await further studies, but what is clear from the investigation by Gallo et al is that the research fires around the role of cigarette smoking in ALS are heating up. Most importantly, the increasing number of large longitudinal investigations of risk factors, combined with the ongoing efforts to identify susceptibility genes, may provide much needed new insights into the possible causes and mechanisms of ALS.

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Vascular Endothelial Growth Factor Gene Transfer for Diabetic Polyneuropathy

Diabetic neuropathy is the most common neuropathy in the industrialized world. The cause of the disorder is poorly understood, which complicates development of effective therapies.[1] Optimum glycemic control decreases the risk for development of peripheral neuropathy,[2,3] but there are not yet any therapies that effectively reverse impaired nerve function. Current pharmacological approaches, therefore, focus primarily on reduction of painful symptoms. In this issue of *Annals,* Ropper and colleagues[4] report the results of a phase 2 randomized trial of intramuscular gene transfer using plasmid vascular endothelial growth factor (VEGF) to treat diabetic neuropathy. VEGF is a proangiogenic growth factor, and the hypothesis underlying Ropper and colleagues'[4] approach is that microvascular ischemia is an important cause of diabetic polyneuropathy.[5,6] In this trial, either VEGF-expressing plasmid or placebo was injected into multiple sites in one leg, and the contralateral leg was used to adjust for changes occurring independent of the therapeutic intervention. The primary end point was improvement in the adjusted sensorimotor symptom score and the trial successfully met this test of efficacy. Although there was an increased incidence of serious