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Neurobiology of Aging 34 (2013) 357.e7–357.e19

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Age of onset of amyotrophic lateral sclerosis is modulated by a locus on 1p34.1

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Received 19 May 2012; received in revised form 26 July 2012; accepted 29 July 2012

Abstract

Amyotrophic lateral sclerosis (ALS) is the third most common adult-onset neurodegenerative disease. Individuals with ALS rapidly progress to paralysis and die from respiratory failure within 3 to 5 years after symptom onset. Epidemiological factors explain only a modest amount of the risk for ALS. However, there is growing evidence of a strong genetic component to both familial and sporadic ALS risk. The International Consortium on Amyotrophic Lateral Sclerosis Genetics was established to bring together existing genome-wide association cohorts and identify sporadic ALS susceptibility and age at symptom onset loci. Here, we report the results of a meta-analysis of the International Consortium on Amyotrophic Lateral Sclerosis Genetics genome-wide association samples, consisting of 4243 ALS cases and 5112 controls from 13 European ancestry cohorts from across the United States and Europe. Eight genomic regions provided evidence of association with ALS, including 9p21.2 (rs3849942, odds ratio [OR] = 1.21; $p = 4.41 \times 10^{-7}$), 17p11.2 (rs7477, OR = 1.30; $p = 2.89 \times 10^{-7}$), and 19p13 (rs12608932, OR = 1.37, $p = 1.29 \times 10^{-7}$). Six genomic regions were associated with age at onset of ALS. The strongest evidence for an age of onset locus was observed at 1p34.1, with comparable evidence at rs3011225 ($R^2_{\text{partial}} = 0.0061$; $p = 6.59 \times 10^{-8}$) and rs803675 ($R^2_{\text{partial}} = 0.0060$; $p = 6.96 \times 10^{-8}$). These associations were consistent across all 13 cohorts. For rs3011225, individuals with at least 1 copy of the minor allele had an earlier average age of onset of over 2 years. Identifying the underlying pathways influencing susceptibility to and age at onset of ALS may provide insight into the pathogenic mechanisms and motivate new pharmacologic targets for this fatal neurodegenerative disease.

Keywords: Amyotrophic lateral sclerosis; Genome-wide association study; Age at onset

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the third most common adult-onset neurodegenerative disease, affecting approximately 10,000 Americans annually (Hirtz et al., 2007). Disease progression is rapid and results in progressive paralysis and death from respiratory failure, usually within 3 to 5 years. The current estimated incidence is between 2.2 and 2.8 per 100,000 (Logroscino et al., 2008; Traynor et al., 1999; Worms, 2001; Zoccolella et al., 2006) with a lifetime prevalence of about 1 in 300 (Johnston et al., 2006). Risk factors for ALS include male gender, increasing age, positive family history of ALS, and smoking (Armon, 2009; Siddique et al., 1998). Males tend to have a slightly earlier age of onset than females (approximately 65 and approximately 67 years of age, respectively) and a male-to-female ratio of approximately 1.6 that varies with age (Manjaly et al., 2010). Approximately 5%–10% of individuals diagnosed with ALS have a family history (i.e., familial ALS), while the remaining 90%–95% do not report a family history and are classified as sporadic ALS (Al-Chalabi and Lewis, 2011; Chiò et al., 2008; Fallis and Hardiman, 2009; Fang et al., 2009; Hanby et al., 2011; Logroscino et al., 2005; Traynor et al., 1999). Of the other posited risk factors, smoking provides the strongest epidemiological factor (Weisskopf et al., 2010); other factors such as exercise, heavy metal and occupational exposures, remain controversial. Taken together, these epidemiologic risk factors do not

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http://dx.doi.org/10.1016/j.neurobiolaging.2012.07.017

explain much of the variation in the risk for ALS, age of onset of motor neuron dysfunction, or survival time once diagnosed with ALS.

The increased familial risk and the identification of genetic variation that increases risk of ALS are consistent with ALS being a genetic trait. Analyses of multiplex families have identified mutations in several genes that are causative for ALS, including but not limited to *SOD1* (Rosen et al., 1993, 1994), *VAPB* (Nishimura et al., 2004), *TARDBP* (Sreedharan et al., 2008), *FUS* (Kwiatkowski et al., 2009; Vance et al., 2009), *OPTN* (Maruyama et al., 2010), *VCP* (Johnson et al., 2010), and *UBQLN2* (Deng et al., 2011). Genome-wide association studies (GWAS) that have been completed for sporadic ALS have identified loci of interest such as *DPP6* (Cronin et al., 2008; van Es et al., 2008), *ITPR2* (van Es et al., 2007), *SUNC1* (Chiò et al., 2009), *UNC13a* (van Es et al., 2009), and 9p21.2 (Laaksovirta et al., 2010; Shatunov et al., 2010; van Es et al., 2009). Among these, only the association signal on chromosome 9p21 has been consistently replicated across studies. Building upon these GWAS results, it was recently reported that a large hexanucleotide repeat expansion of the *C9orf72* gene underlies this locus in a portion of both sporadic and familial ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Thus, the *C9orf72* result underscores the importance of well-powered GWAS in ALS.

Efforts to find genetic factors that influence ALS phenotypes are also under way, but have yet to yield unambiguous results. Variants within *KIFAP3* have been implicated in modifying patient survival time from diagnosis of ALS (Landers et al., 2009), but these variants were not replicated in an independent cohort (Traynor et al., 2010). Similarly *CHGB*, *TXNRD1*, *APOE*, and *VEGF* variants and a 50-base pair promoter deletion of *SOD1*, have been associated with modifying age of ALS onset (Gros-Louis et al., 2009 [*CHGB*], Mitchell et al., 2009 [*TXNRD1*], Li et al., 2004 [*APOE*], Oosthuyse et al., 2001 [*VEGF*]). Age of ALS onset shows evidence for heritability in studies of multiple families with *SOD1* mutations from a single founder (Fogh et al., 2007), but none of these loci are considered established. The amyotrophic lateral sclerosis online database (ALSOD; alsod.iop.kcl.ac.uk/) and the ALSGene database (www.alsgene.org/) provide an up-to-date summary of ALS genetic risk factors for familial and sporadic ALS (Lill et al., 2011; Wroe et al., 2008).

There are multiple plausible reasons for the modest number of established sporadic ALS, age of onset, and susceptibility loci. These reasons parallel the relatively modest number of weak epidemiologic risk factors that are considered established. One might hypothesize that the genetic influences on sporadic ALS are weak. However, the lack of strong epidemiologic factors, the existence of known genetic influences on familial ALS, twin studies (Al-Chalabi et al., 2010), and the existence of sporadic ALS predisposing variants suggest that genetic factors exist. Alternatively, one might hypothesize that the lack of established associations may be the result of modest statistical power, given that ALS is a relatively rare disease and most loci likely have modest effects.

The International Consortium on Amyotrophic Lateral Sclerosis Genetics (ALSGEN; https://alsgen.phs.wfubmc.edu/public/index.cfm) was established to combine resources to maximize the power to identify ALS susceptibility loci and genetic variation that influences age at symptom onset. Here, we report the results of a joint and meta-analysis of the ALSGEN GWAS samples focused on predisposition to ALS and ALS age of onset.

2. Methods

2.1. Subjects

Samples were received from a total of 13 sources spanning the United States and Europe, including Belgium, France, the Netherlands, Ireland, Italy, Sweden, and the United Kingdom, with the Netherlands and Ireland each contributing 2 separate cohorts (Supplementary Table 1). All cases met the El Escorial criteria for probable or definite ALS. Controls were received from all sources except the French cohort. With the exception of samples from Northwestern University, all samples have been described in previous publications (Chiò et al., 2009; Cronin et al., 2008; Landers et al., 2009; Schymick et al., 2007; Traynor et al., 2010; Valdmanis et al., 2007; van Es et al., 2007, van Es et al.,2009). The Northwestern sporadic ALS are age and ethnicity matched cases are from the Neurologic Diseases Registry, with spousal or community controls. All samples were of self-reported Caucasian ethnicity and of European or European-American ancestry.

2.2. Genotyping

All of the contributed samples were genotyped on 1 of the Illumina (San Diego, CA, USA) single nucleotide polymorphism (SNP) chips (see Supplementary Table 1); 254,145 SNPs common across the various SNP genotyping arrays and that passed quality control were analyzed in these cohorts.

2.3. Statistical analysis

A principal-component analysis of all 254,145 autosomal SNPs that pass the highest standards of quality control (i.e., low missingness, no differential missingness between cases and controls, no departure from Hardy–Weinberg Equilibrium expectations, relatedness, gender ambiguities, heterozygosity outliers, samples with low call rates ($<$ 95%) and samples contributing investigators excluded in their previously published analyses) was computed. Two principal components were identified that reduced the overall inflation factor and were included as covariates in all subsequent modeling. Additional principal components did not further reduce inflation factor nor substantively change