The role of single-nucleotide variants of the energy metabolism-linked genes SIRT3, PPARGC1A and APOE in amyotrophic lateral sclerosis risk

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Amyotrophic lateral sclerosis (ALS) is a multifactorial disease, possibly with contributions from genetics and lifestyle. We examined variants in genes relevant to energy metabolism and physical activity in a case-control association study, with the aim of assessing genetics and physical activity as contributors to ALS risk. A well-characterized sample of Italian ALS patients (101) and controls (101) from the EURALS Consortium underwent a questionnaire interview on demographic, physical and other lifestyle habits, and venipuncture for DNA extraction. The genes selected were sirtuin 3 (SIRT3), peroxisome proliferator-activated receptor-γ coactivator-1α (PPARGC1A) and apolipoprotein E (APOE). Genetic studies suggested, for the first time, a protective role of the SIRT3 single-nucleotide polymorphism rs4980329 in ALS risk, and a contribution of the APOE-ε2 allele, which was more frequent in ALS patients than in controls. A joint analysis coupling genetic data and sporting activity revealed opposite roles of APOE-ε2 and SIRT3 rs3825075, the former being more frequent in physically active ALS patients and the latter more frequent in physically inactive patients. These findings suggest a contribution to ALS risk of genetic and environmental factors involved in energy metabolism, and stress the importance of a multifactorial analysis for evaluating this risk.

Key words: amyotrophic lateral sclerosis, physical activity, sirtuin 3, apolipoprotein E, PPARGC1A

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a multifactorial neurodegenerative disease of unknown etiology (Peters et al., 2015). Many genetic and lifestyle factors can contribute to its onset. Particular attention has been paid to physical activity and sports as possible risk modifiers (Beghi, 2013). The contribution to ALS risk of physical activity may be further mediated by individual genetic background, which might exacerbate or alleviate the potential neurodegenerative consequences of sport (e.g., the increase of free radicals and ensuing oxidative stress) (Slattery et al., 2015). Human sirtuins (SIRTs) are enzymes with NAD+-dependent histone deacetylase activity, and are involved both in neurodegeneration and in energy metabolism. SIRTs are encoded by seven genes that contribute to multiple physiological and pathological pathways (Albani et al., 2010; Polito et al., 2013). Three SIRTs are localized in the mitochondria (SIRT3, 4 and 5), and SIRT3 has been linked to oxidative stress control (Sundaresan et al., 2008; Kwon et al., 2015; Xie et al., 2016). Increased expression of the SIRT3 gene has a pro-survival function in mice (Benigni et al., 2009), and a putative positive contribution to human longevity (Rose et al., 2003; Albani et al., 2014; Barger et al., 2015).

Another gene relevant to energy metabolism but also to neurodegeneration is apolipoprotein E (APOE), a well-known risk factor for Alzheimer's disease and vascular dementia when present as the APOE-ε4 allele (Saunders et al., 1993; Slooter et al., 1997); conversely, the presence of the APOE-ε2 allele seems to correlate with a protective
phenotype in these neurodegenerative disorders (Talbot et al., 1994; Berge et al., 2014; Serrano-Pozo et al., 2015).

The effect of the APOE genotype on susceptibility to other neurodegenerative diseases is still controversial. For instance, an association between frontotemporal lobar degeneration and the APOE-ε4 allele has been reported but not always replicated (Minthon et al., 1997; Ingelson et al., 2001; Ji et al., 2013). As regards a specific role in ALS, many studies have been published and the overall effect of the APOE-ε4 allele alone seems non-significant (Mui et al., 1995; Govone et al., 2014), while the possible protective role of the APOE-ε2 form has been poorly explored (Buée et al., 1996).

Peroxisome proliferator-activated receptor (PPAR)-gamma coactivator1 alpha (PPARGC1A, also known as PGC-1α) is a nuclear transcriptional coactivator that plays a pivotal role in metabolic processes including mitochondrial biogenesis, thermogenesis, respiration, insulin secretion and gluconeogenesis (Houten and Auwerx, 2004; Baar, 2014). PPARGC1A regulates muscle fiber development and, importantly, its transcription is stimulated by physical exercise (Agudelo et al., 2014; Popov et al., 2015; Tiano et al., 2015). A genetic study reported an association between PPARGC1A variants and aerobic capacity (Nishida et al., 2015). PPARGC1A has been linked to molecular pathways involved in several neurological disorders, including Alzheimer’s, Parkinson’s and Huntington’s disease, ALS, multiple sclerosis, and stroke (Cui et al., 2006; Róna-Vörös and Weydt, 2010; Ghosh et al., 2015). Our discovery phase case-control association study was designed to assess the contribution of physical activity and genetic variants of SIRT3, APOE and PPARGC1A, alone or in combination, to sporadic ALS risk.

MATERIALS AND METHODS

Patients Cases (101: bulbar/generalized onset, 29; spinal onset, 68; other onset/unknown, 4) and controls (101) included in the present study were a subgroup of a multicenter study. They were selected for the availability of both genomic DNA (gDNA) and data on physical activity. In detail, starting on 1 February 2008 and ending on 30 April 2012, 652 cases were recruited from among patients with newly diagnosed ALS who were attending centers participating in a population-based study investigating the relationship between ALS and physical activities, from the EURALS Consortium (Pupillo et al., 2014). Patients were enrolled by neurologists during ambulatory visits or in hospital in five European countries, including Italy. Next, 1,166 controls, matched for sex, age (± 2.5 years) and residency, were enrolled by local general practitioners during routine visits. After giving informed consent, patients and controls were interviewed by a trained investigator at each center, using an ad hoc structured questionnaire. Baseline demographic and clinical data (date of birth, sex, years of education, weight, height, body mass index [BMI], residency) were collected for cases and controls.

Physical activity A detailed history of patients’ and controls’ occupation was collected, including job description, date of start and cessation, level of physical activity and hours spent on physical activity per month. A detailed lifetime history of each sport was also collected, including type, date of start and cessation, level of physical activity and hours spent per month on each sport. Physical activity was related to work, sport, or both. Organized sport was any sport practiced for at least one year by joining a sporting association and participating in official competitions. Professional sport was any sport practiced for at least one year, intended as the

| Table 1. Main demographic and clinical characteristics of ALS cases and controls |
| --- |
| Variable | Category | ALS | Controls | P value |
| --- |
| Sex | Women | 39 | 38.6 | 39 | 38.6 | 1.0000 |
| | Men | 62 | 61.4 | 62 | 61.4 |
| Age (years) | < 55 | 18 | 17.8 | 15 | 14.9 | 0.6650 |
| | 55–64 | 32 | 31.7 | 29 | 28.7 |
| | 65–74 | 33 | 32.7 | 32 | 31.7 |
| | ≥ 75 | 18 | 17.8 | 25 | 24.7 |
| Education (years) | < 6 | 30 | 29.7 | 27 | 27.3 |
| | 6–9 | 23 | 22.8 | 21 | 21.2 |
| | 9–14 | 36 | 35.6 | 24 | 24.2 | 0.0384 |
| | ≥ 15 | 12 | 11.9 | 27 | 27.3 |
| | NS | 0 | 2 |
| BMI | < 18.5 | 8 | 7.9 | 2 | 2.1 | 0.0101 |
| | 18.5–24 | 58 | 57.4 | 44 | 44.4 |
| | ≥ 25 | 35 | 34.7 | 53 | 53.5 |
| | NS | 0 | 2 |
| Interviewee | Proxy | 16 | 15.8 | 6 | 6.1 | 0.0288 |
| | Proxy | 6 | 5.9 | 1 | 1.0 |
| Traumatic injury | No | 28 | 27.7 | 32 | 31.7 | 0.5380 |
| | Yes | 73 | 72.3 | 69 | 68.3 |
| Coffee | Yes | 66 | 77.6 | 89 | 88.1 | 0.0563 |
| | No | 19 | 22.4 | 12 | 11.9 |
| Alcohol | Yes | 66 | 65.4 | 56 | 55.4 | 0.1503 |
| | No | 35 | 34.7 | 45 | 44.6 |
| Smoking | Yes | 62 | 61.4 | 39 | 38.6 | 0.0012 |
| | No | 39 | 38.6 | 62 | 61.4 |
| NS: not stated. |
main occupation. *Amateur sport* was any sport other than organized or professional. *Leisure activities* were any recreational activity done at least weekly; leisure activities were pooled with amateur sports. Previous exposure to traumatic events (yes/no, with number and details) was also investigated, together with drug, coffee, alcohol and tobacco use.

**DNA extraction and genotyping** Patients and controls who gave their informed consent for genetic testing were subsequently asked to give blood samples. Fast-

ing peripheral blood samples (30 ml) were collected by venipuncture and stored at −80 °C. Genomic DNA was extracted using an automated nucleic acid extractor (Maxwell, Promega, Madison, WI, USA), and checked for concentration and quality with a UV spectrophotometer.
We assessed a set of single-nucleotide polymorphisms (SNPs) from the genes \textit{SIRT3}, \textit{APOE} and \textit{PPARGC1A}. \textit{SIRT3}-selected SNPs were rs536715, rs4980329 and rs3825075, which had previously been investigated in association with neurodegeneration (Polito et al., 2013). \textit{PPARGC1A} rs8192678, rs4235308 and rs2946385 were previously reported as tag-SNPs spanning the entire \textit{PPARGC1A} gene (Lai et al., 2008), while \textit{APOE} rs7412 and rs429358 were selected to assess the locus genotype of \textit{APOE}, which is involved in lipid metabolism and neurodegeneration (Mui et al., 1995; Buée et al., 1996; Minthon et al., 1997; Ingelson et al., 2001; Ji et al., 2013; Govone et al., 2014). A gDNA sample (about 20 ng) was used for allelic discrimination assay with a real-time PCR apparatus and TaqMan technology according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). A quality control of the generated data was performed by a second genotype assessment in a random sample (11 cases and 11 controls) representative of all of the included subjects. We found full accordance with the previous results (100% replication rate).

### Statistical analysis

The main features of ALS cases and their controls were described using counts and percentages, and compared using the chi-square test or Fish-

| SIRT3 rs536715 | ALS | Controls | P value |
|----------------|-----|----------|--------|
| CC             | 42  | 41.6     | 41     | 40.6  | 0.9565 |
| TC             | 24  | 23.8     | 23     | 22.8  |        |
| TT             | 35  | 34.6     | 37     | 36.6  |        |

| PPARGC1A rs8192678 | ALS | Controls | P value |
|---------------------|-----|----------|--------|
| CC                  | 43  | 42.6     | 46     | 45.5  |        |
| TC                  | 44  | 43.5     | 41     | 40.6  | 0.3607 |
| TT                  | 6   | 5.9      | 8      | 7.9   |        |

| PPARGC1A rs4235308 | ALS | Controls | P value |
|---------------------|-----|----------|--------|
| CC                  | 43  | 42.6     | 46     | 45.5  |        |
| TC                  | 44  | 43.5     | 41     | 40.6  | 0.9017 |
| TT                  | 14  | 13.9     | 14     | 13.9  |        |

| PPARGC1A rs2946385 | ALS | Controls | P value |
|---------------------|-----|----------|--------|
| GG                  | 35  | 35.0     | 37     | 36.6  |        |
| GT                  | 39  | 38.0     | 47     | 46.5  | 0.1943 |
| TT                  | 27  | 27.0     | 17     | 16.8  |        |

Table 4. \textit{SIRT3} and \textit{PPARGC1A} genotype distribution in ALS cases and controls after stratification by sex

| Females         | ALS | Controls | P value |
|-----------------|-----|----------|--------|
| SIRT3 rs536715  |     |          |        |
| CC              | 17  | 43.6     | 19     | 48.8  |        |
| TC              | 11  | 28.2     | 10     | 25.6  | 0.9020 |
| TT              | 11  | 28.2     | 10     | 25.6  |        |

| PPARGC1A rs8192678 | ALS | Controls | P value |
|---------------------|-----|----------|--------|
| CC                  | 14  | 35.9     | 19     | 48.7  |        |
| TC                  | 20  | 51.3     | 15     | 38.5  | 0.0479 |
| TT                  | 5   | 12.8     | 5      | 12.8  |        |

| Males            | ALS | Controls | P value |
|------------------|-----|----------|--------|
| SIRT3 rs536715   |     |          |        |
| CC              | 17  | 43.6     | 19     | 48.8  |        |
| TC              | 11  | 28.2     | 10     | 25.6  | 0.9020 |
| TT              | 11  | 28.2     | 10     | 25.6  |        |

| PPARGC1A rs8192678 | ALS | Controls | P value |
|---------------------|-----|----------|--------|
| CC                  | 14  | 35.9     | 19     | 48.7  | 0.0479 |
| TC                  | 20  | 51.3     | 15     | 38.5  | 0.0479 |
| TT                  | 5   | 12.8     | 5      | 12.8  |        |

| PPARGC1A rs4235308 | ALS | Controls | P value |
|---------------------|-----|----------|--------|
| CC                  | 10  | 26.3     | 6      | 15.4  | 9.7    |
| TC                  | 20  | 52.6     | 16     | 41.0  | 0.0967 |
| TT                  | 8   | 21.1     | 17     | 43.6  | 0.6940 |

| PPARGC1A rs2946385 | ALS | Controls | P value |
|---------------------|-----|----------|--------|
| GG                  | 9   | 23.1     | 15     | 38.5  | 0.0129 |
| GT                  | 12  | 30.8     | 18     | 46.1  |        |
| TT                  | 18  | 46.1     | 6      | 15.4  | 0.0129 |

Table 3. Genotype distribution of \textit{SIRT3} and \textit{PPARGC1A}
ALS, physical activity and genetic background

The relationship between physical activity and ALS was evaluated using univariate and multivariate conditional logistic regression models. Seven different aspects of exposure to physical activity were considered: I. physical activity (yes/no); II. type of physical activity (none/leisure/work/sport/work & sport); III. sport (yes/no); IV. sport category (none/amateur/organized/professional); V. duration of physical activity (in quartiles); VI. duration of sport (in quartiles); VII. age at starting sport (<15/15–34/34–55/55+). Multivariate models were adjusted for interviewee (patient/control or proxy), traumatic injuries, smoking habits, and coffee and alcohol consumption. Results are presented as odds ratios (ORs) and adjusted odds ratios (adjORs) with 95% confidence intervals (95% CI).

RESULTS

The main demographic and clinical characteristics of ALS cases and controls are reported in Table 1. The two groups were balanced for sex and age, and the features with significant differences were education, BMI and smoking habits. We also observed a significant difference in the interviewees.

Next, we compared the physical activity of ALS cases and controls. ALS risk was inversely associated with overall and sport-related physical activity, but the association lost significance after adjustment for interviewee, traumatic injury, coffee and alcohol consumption, and smoking habit (Table 2).

We then assessed the genetic contribution to ALS risk of SIRT3, PPARGC1A and APOE. The genotypic distribution of SIRT3 and PPARGC1A variants is shown in Table 3. We found a nominal (but not Bonferroni’s-corrected) difference in genotypic distribution between ALS and controls for SIRT3 rs4980329, and for this SNP the T-allele frequency was lower in ALS patients (T-allele frequency in ALS vs. controls: 40.5% and 51.0%, respectively, \( P = 0.0904 \)). No association was seen for PPARGC1A.

SIRT3 rs4980329 stratified by sex did not give sig-

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Table 5. APOE genotype distribution in ALS cases and controls

|        | ALS   | Controls | \( P \) value | ALS   | Controls | \( P \) value |
|--------|-------|----------|--------------|-------|----------|--------------|
|        | No. % | No. %    |              | No. % | No. %    |              |
| c2/c2  | 1 1.0 | 0 0.0    |              | c4−   | 71 71.7  | 70 28.3      | 0.7086 |
| c2/c3  | 14 14.2| 6 5.9    |              | c4+   | 28 28.3  | 31 30.7      |        |
| c2/c4  | 5 5.0 | 1 1.0    |              |       |          |              | 0.1034 |
| c3/c3  | 56 56.6| 64 63.4  |              |       |          |              |        |
| c3/c4  | 22 22.2| 28 27.7  |              | c2−   | 79 79.8  | 94 93.1      | 0.0006 |
| c4/c4  | 1 1.0 | 2 2.0    |              | c2+   | 20 20.2  | 7 9.9        |        |

Females

|        | ALS   | Controls | \( P \) value | ALS   | Controls | \( P \) value |
|--------|-------|----------|--------------|-------|----------|--------------|
|        | No. % | No. %    |              | No. % | No. %    |              |
| c2/c2  | 1 2.6 | 0 0.0    |              | c4−   | 29 74.1  | 23 59.0      | 0.1495 |
| c2/c3  | 6 15.4| 1 2.6    |              | c4+   | 10 25.6  | 16 41.0      |        |
| c2/c4  | 0 0.0 | 0 0.0    |              |       |          |              | 0.0904 |
| c3/c3  | 22 56.4| 22 56.4  |              |       |          |              |        |
| c3/c4  | 10 25.6| 16 41.0  |              | c2−   | 32 81.1  | 38 97.4      | 0.0564 |
| c4/c4  | 0 0.0 | 0 0.0    |              | c2+   | 7 17.9   | 1 2.6        |        |

Males

|        | ALS   | Controls | \( P \) value | ALS   | Controls | \( P \) value |
|--------|-------|----------|--------------|-------|----------|--------------|
|        | No. % | No. %    |              | No. % | No. %    |              |
| c2/c2  | 0 0.0 | 0 0.0    |              | c4−   | 42 70.0  | 47 75.8      | 0.4704 |
| c2/c3  | 8 13.3| 5 8.1    |              | c4+   | 18 30.0  | 15 24.2      |        |
| c2/c4  | 5 8.3 | 1 1.6    |              |       |          |              | 0.3422 |
| c3/c3  | 34 56.7| 42 67.7  |              | c2−   | 47 78.3  | 56 90.3      | 0.0679 |
| c4/c4  | 1 1.7 | 2 3.2    |              | c2+   | 13 21.7  | 6 9.7        |        |
significant results at the Bonferroni’s-corrected level, even though this variant had a different distribution in females, at a nominal level of significance ($P = 0.0398$). However, $PPARGC1A$ rs2946385 showed a different genotypic distribution in females, with an increase of the T-variant in ALS. No difference was detectable for males (Table 4).

The $APOE$ genotype distribution did not differ in ALS and controls, for the whole group or after stratification for

| Table 6. $SIRT3$ and $PPARGC1A$ genotype distribution by sport in ALS cases and controls |
|-----------------------------------------------|
|                                          | No sport | Sport | |
|                                          | ALS      | Controls | $P$ value | ALS | Controls | $P$ value |
|                                          | No. %    | No. %    |            | No. % | No. %    |            |
| $SIRT3$ rs536715                        | CC 23    | 41.1     | 9 29.0     | 19   | 42.2     | 32 45.7    |
|                                          | TC 11    | 19.6     | 7 22.6     | 13   | 28.9     | 16 22.9    | 0.5331
|                                          | TT 22    | 39.3     | 15 48.4    | 13   | 28.9     | 22 31.4    |
| $SIRT3$ rs4980329                       | CC 20    | 36.4     | 13 41.9    | 16   | 35.6     | 20 28.6    |
|                                          | TC 29    | 52.7     | 10 32.3    | 18   | 40.0     | 24 34.3    | 0.0956
|                                          | TT 6     | 10.9     | 8 25.8     | 11   | 24.4     | 26 37.1    |
| $SIRT3$ rs3825075                       | CC 25    | 44.6     | 24 77.4    | 19   | 42.2     | 28 40.0    | 0.048
|                                          | TC 29    | 51.8     | 6 19.4     | 22   | 48.9     | 35 50.0    | 0.0048
|                                          | TT 6     | 10.9     | 1 3.2      | 4    | 8.9      | 7 10.0     |
| Allelic count                           | C 79     | 70.5     | 54 87.1    | 0.014
|                                          | T 33     | 29.5     | 8 12.9     |
| $PPARGC1A$ rs8192678                    | CC 26    | 46.4     | 15 48.4    | 17   | 37.8     | 31 44.3    |
|                                          | TC 26    | 46.4     | 12 38.7    | 18   | 40.0     | 29 41.4    | 0.6376
|                                          | TT 4     | 7.2      | 4 12.9     | 10   | 22.2     | 10 14.3    |
| $PPARGC1A$ rs4235308                    | CC 12    | 21.4     | 12.9 12.9  | 7    | 15.9     | 8 11.4     |
|                                          | TC 30    | 53.6     | 51.6 51.6  | 23   | 52.3     | 36 51.4    | 0.4512
|                                          | TT 14    | 25.0     | 35.5 35.5  | 14   | 31.8     | 26 37.1    |
| $PPARGC1A$ rs2946385                    | GG 20    | 35.8     | 13 41.9    | 15   | 34.1     | 24 34.3    |
|                                          | GT 18    | 32.1     | 11 35.5    | 20   | 45.5     | 36 51.4    | 0.6359
|                                          | TT 18    | 32.1     | 7 22.6     | 9    | 20.4     | 10 14.3    |

*P value for the allelic distribution of $SIRT3$ rs3825075 between ALS and Controls under the “No sport” group (chi-square test).

| Table 7. $APOE$ genotype distribution by sport in ALS cases and controls |
|-----------------------------------------------|
|                                          | No sport | Sport | |
|                                          | ALS      | Controls | $P$ value | ALS | Controls | $P$ value |
|                                          | No. %    | No. %    |            | No. % | No. %    |            |
| $e2/e2$                                  | 1        | 1.8      | 0 0.0      | 45   | 80.4     | 23 74.2    | 0.5052
|                                          | 6        | 10.7     | 1 3.2      | 11   | 19.6     | 8 25.8     |
| $e2/e3$                                  | 1        | 1.8      | 0 0.0      | 38   | 67.9     | 22 71.0    | 0.4719
|                                          | 10       | 17.8     | 7 22.6     | 4    | 0.0      | 1 3.2      |
| $e3/e3$                                  | 0.0      | 0.0      | 1 3.2      | 0    | 0.0      | 1 3.2      |
| $e4/e4$                                  | 0        | 0.0      | 0 0.0      | 4    | 9.3      | 1 1.4      | 0.0531
|                                          | 8        | 18.6     | 5 7.2      | 12   | 27.9     | 21 30.0    | 0.0086
|                                          | 4        | 9.3      | 1 1.4      | 1    | 2.3      | 1 1.4      |

*P value for the allelic distribution of $SIRT3$ rs3825075 between ALS and Controls under the “No sport” group (chi-square test).
males and females (Table 5). When we divided the population according to the presence of at least one APOE-ε4 allele, there was still no difference. However, a similar stratification according to the presence of the APOE-ε2 allele indicated a higher frequency of this allele in ALS, not influenced by sex (Table 5).

We also tried to correlate the genetic contribution to ALS risk of SIRT3, PPARGC1A and APOE when comparing bulbar/generalized onset (29) to spinal onset (68). In this case, due to the limited sample size, we did not divide the population according to sex. The only Bonferroni-corrected association at the genotypic level was for PPARGC1A rs4235308 ($P = 0.012$). For this variant, the C-allele frequency predominated in patients with bulbar/generalized onset (60.0% vs. 38.0%, $P = 0.004$).

Finally, we tried to correlate the genetic variants, physical activity and ALS risk. After stratification of the population according to sport, we found that the SIRT3 variant rs3825075 was differently distributed in physically active and inactive individuals at the genotype level. In particular, for physically inactive subjects the T-allele was more frequent in ALS than in controls (T-allele frequency 29.5% vs. 12.9%, $P = 0.0014$). PPARGC1A analysis gave no significant association with ALS (Table 6).

The difference in APOE genotype distribution was not significant after stratification by physical activity. However, in the physical activity group the APOE-ε2 allele was significantly more frequent in ALS patients than in controls (ε2 allele frequency in ALS vs. controls of 27.9% and 8.6%, respectively, $P = 0.0086$) (Table 7).

**DISCUSSION**

Sporadic ALS is a multifactorial disorder with genetic and environmental components, each of them difficult to replicate as a risk modifier. This is the case for physical activity, where the overall evidence does not suggest a clear relevance (Beghi, 2013; Hamidou et al., 2014). However, genomic variability in genes important in energy metabolism may contribute to the individual response to physical activity as an ALS risk modulator. Thus, in the present study, we tried to develop an innovative strategy and assessed both physical activity and single-nucleotide polymorphisms (SNPs) as risk factors for sporadic ALS in a sample of cases and controls from a multicenter collaborative investigation. A previous case-control study found an inverse correlation between ALS occurrence and overall physical activity, work-related physical activity, duration of physical activity, cumulative Metabolic Equivalent of Task (MET) scores and sport-related physical activity in men, but not women (Pupillo et al., 2013). Therefore, we selected genes having a role in mitochondrial function and energy, glucose homeostasis and lipid metabolism.

The genetic analysis of SIRT3 polymorphisms gave some indications for the rs4980329 T-variant, which was at the limit of Bonferroni’s-corrected significance ($P = 0.0195$) and less frequent in cases than in controls (with a tendency to a reduction in females after stratification by sex). This intronic SNP has never been associated with ALS, but it has been reported in the context of neurodegeneration or longevity, and in a study assessing genetic predisposition to carotid plaque deposition (Polito et al., 2013; Albani et al., 2014; Dong et al., 2015). Due to the lack of suitable biological materials (e.g., blood lymphocytes), we were not able to assess if the rs4980329 T-variant was functionally active and able to affect peripheral SIRT3 expression. Of relevance, a previous study reported that SIRT3 overexpression was protective in a model of ALS, thus reinforcing the involvement of this sirtuin in ALS (Song et al., 2013).

According to published reports, the second gene (PPARGC1A) had a clear role in energy metabolism during physical activity, but in our analysis we only found a significant effect of rs2946385 after sex stratification that was absent (even as a trend) in the whole population; this effect may be a false-positive finding.

The APOE gene is not a novel risk factor for ALS, but we included it for an independent confirmation and because of its role in fatty acid metabolism, also relevant for energy homeostasis and a possible interaction with physical activity (Craft et al., 1999; Chouinard-Watkins and Plourde, 2014). Our data confirmed the lack of association between the APOE variant ε4 and ALS, whereas we found an association between the disease and the APOE-ε2 allele, which is generally considered neuroprotective (Talbot et al., 1994; Berge et al., 2014; Serrano-Pozo et al., 2015). In our case, its frequency was increased in ALS, with no sex-specific effect.

There are reports casting doubt on the protective role of the APOE-ε2 allele. For instance, van Duijn et al. reported a detrimental action of the APOE-ε2 allele in Alzheimer’s disease, particularly on survival (van Duijn et al., 1995), and, in accordance with our findings, Chiò et al. highlighted an association between this allele and the risk of frontotemporal dementia in ALS (Chiò et al., 2016). Overall, data on the specific role of the APOE-ε2 allele in ALS are very limited (Buée et al., 1996; Chiò et al., 2016), so our contribution may refresh the discussion on this genetic variant.

When we compared bulbar/generalized and spinal-onset ALS cases for their genotypes, we found a different PPARGC1A rs2946385 profile. This is a novel finding that needs confirmation in larger datasets, because there are no comparative published reports.

As previously stated, our first aim was to detect any interaction between physical activity and genetic variants in ALS risk. To this end, we stratified the population according to sport and genetic background in the selected loci. Interestingly, we found an association for a SIRT3 variant (rs3825075) and for the APOE-ε2
allele. The former was more frequent in ALS patients not practicing sports, while the APOE-e2 allele was more frequent in physically active ALS cases. No association for PPARGC1A was detected.

Despite the limited sample size, which puts the study at risk of false-negative findings, our approach, namely trying to incorporate genetic and environmental factors for ALS risk, does seem promising and worthy of replication in larger datasets to address the roles of SIRT3 and APOE-e2 in ALS risk, alone and in combination with environmental factors.

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CONFLICT OF INTEREST DISCLOSURE

The authors have no conflict to disclose.

ETHICAL APPROVAL

All procedures in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments.

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