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BRIEF COMMUNICATION

PON1 is a disease modifier gene in amyotrophic lateral sclerosis: association of the Q192R polymorphism with bulbar onset and reduced survival

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Abstract

Introduction Previous studies have associated single-nucleotide polymorphisms (SNPs) in the gene encoding the detoxifying enzyme paraoxonase 1 (*PON1*) to the risk of sporadic ALS. Here, we aimed to assess the role of the coding rs662 (Q192R) SNP as a modifier of ALS phenotype.

Materials and methods We genotyped a cohort of 409 patients diagnosed with ALS at our Center between 2002 and 2009 (269 males and 140 females; mean age at onset, 58.3 ± 37.5 years).

Results We found *PON1* to be a disease modifier gene in ALS, with the minor allele G associated both with bulbar onset (30.9% vs. 24.6%, $p = 0.013$) and independently with reduced survival (OR = 1.38, $p = 0.012$) under a dominant model. No association was found with gender or age at onset.

Discussion As this SNP is known to modify the detoxifying activity of paraxonase 1 with respect to different substrates as well as other activities of the protein, we hypothesize that the identified association might reflect specific motor neuron vulnerability to certain exogenous toxic substances metabolized less efficiently by the 192R alloenzyme, or to detrimental endogenous pathophysiological processes such as oxidative stress. Further exploration of this possible metabolic susceptibility could deepen our knowledge of ALS pathomechanisms.

Keywords ALS · Motor neurons · Paraoxonase · *PON1* · SNP · Toxicity

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons and leading to death from progressive paralysis in a median time of 3–5 years [1]. ALS displays wide variation in phenotype and survival, with older age at onset and bulbar onset representing established negative prognostic factors [2]. The vast majority of ALS cases are sporadic (sALS), whereas 5–10% are familial (fALS), caused by genetic mutations. The etiology of sALS is not clearly understood, presumably involving an interaction between genetic and environmental factors [1].

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Genetic susceptibility to sALS has been the subject of intensive investigation, including that on the *PON1–3* genes encoding the paraoxonases. These are esterase enzymes with antioxidative properties protecting low-density lipoproteins (LDLs) from lipid peroxidation; PON1 is also able to hydrolyze toxic compounds such as organophosphate pesticides and nerve gases. PON1 and PON3 are produced in the liver and found in circulating high-density lipoproteins (HDLs), whereas PON2 is expressed in many tissues and located intracellularly [3]. *PON* genes were selected as candidate genes in genetic susceptibility studies on the basis of epidemiological data suggesting a link between exposure to organophosphate pesticides or nerve gases and sALS risk [4, 5], as well as because of the known pathogenic relevance of oxidative stress in ALS [6].

Indeed, an association between *PON* variants and sALS susceptibility has been suggested by previous work, albeit inconsistently [7–14]. Slowik et al. compared 185 sALS patients and 437 healthy controls and found the minor allele R of the coding Q192R polymorphism (corresponding to an arginine residue at position 192) to be associated with sALS [8]. The association was confirmed using a recessive model by Wills et al. [13]. Other studies found associations with sALS for another coding SNP in *PON1* (L55M) and an intronic variant in *PON3* [7], an intronic haplotype in *PON1* [9], a haplotype in *PON2* [14], and a haploblock spanning *PON2* and *PON3* [11]. However, two meta-analyses did not confirm an association of *PON* polymorphisms with risk of sALS [10, 12]. On the other hand, *PON1–3* rare variants were found in a small portion of fALS and sALS patients [15].

Given these premises, we aimed to investigate whether *PON1* could also act as a disease modifier gene in ALS. We focused on the rs662 (Q192R) SNP because of its previously suggested association with sALS risk [8, 13] and its influence on catalytic activity of the encoded enzyme [3]. Therefore, we conducted a case-only study on a cohort of ALS patients to investigate whether rs662 is associated with specific phenotypic or prognostic variables of the disease.

Table 1 Demographic and clinical characteristics of the study population

Total number of patients		409
Gender: male, female	No.	269, 140
	%	65.8, 34.2
Age at onset (years, mean ± SD)		58.3 ± 37.5
Site of onset: bulbar, spinal	No.	102, 307
	%	24.9, 75.1
Death or tracheostomy	No.	250
	%	61.1%
Survival (months, mean ± SD)		47.5 ± 32

Materials and methods

Study population

We studied a cohort of 409 consecutive Italian ALS patients diagnosed according to the revised El Escorial criteria [16] at our ALS Center. The demographic and clinical characteristics of the study population are summarized in Table 1.

DNA extraction and SNP genotyping

Genomic DNA was extracted from peripheral blood using a commercial kit according to the manufacturer's protocol (Wizard Genomic DNA Purification Kit, Promega, Madison, WI) and quantified by a NanoDrop 2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Genotyping was conducted using Human 660W-Quad BeadChips on HiScan platform (Illumina, San Diego, CA). Data generated on Illumina microarray platform were analyzed by GenomeStudio software. Genotype data of SNP rs662 in the *PON1* gene were extrapolated for all selected patients.

Statistical analyses

All statistical analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 23.0, IBM Corporation, Armonk, NY). Comparison of the three rs662 SNP genotypes was performed using three different genetic models: dominant (AA vs. (GA + GG)), recessive ((AA + GA) vs. GG), and additive (AA vs. GA vs. GG), where A is the major allele and G is the minor allele. Gender and site of onset were compared using the chi-squared test. Age at onset was compared using Student's *t* test. The effect of rs662 on survival was estimated by univariate Kaplan-Meier survival analysis with log-rank comparison test and multivariate Cox regression model analysis, taking gender, age at onset, and site of onset as covariates. Censoring was applied for patients alive at last follow-up. A p value < 0.05 was considered as statistically significant.

Results

PON1 rs662 polymorphism was not associated with age at onset. On the contrary, a significant association with site of onset was found under the dominant model, with over-representation of bulbar onset among patients carrying one or two copies of the minor allele G corresponding to an arginine residue at amino acid position 192 of the protein (30.9% in (GA + GG) vs. 24.6% in AA; $p = 0.013$). Similarly, rs662 was also associated with survival, which was shorter for GA and GG genotypes in comparison with AA (44.5 vs. 50.6 months; $p = 0.016$) (Fig. 1).

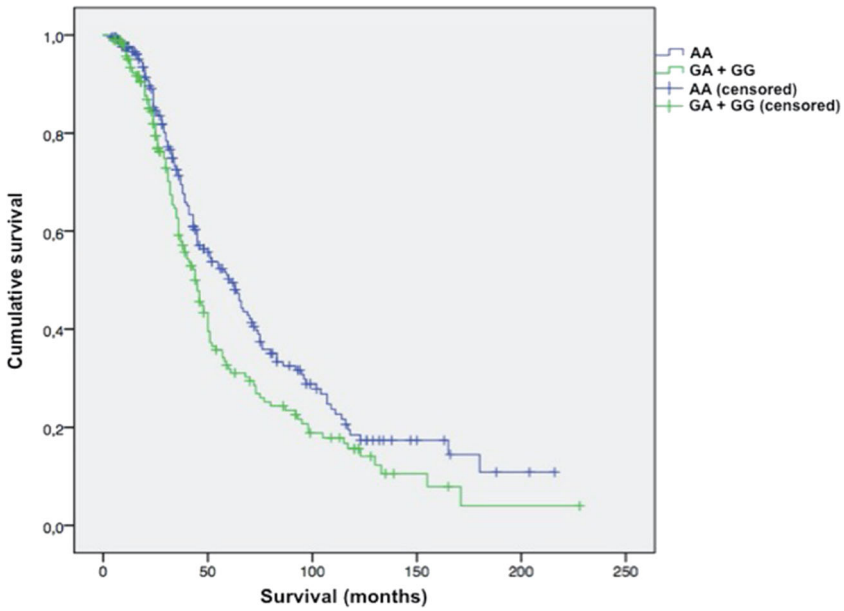

Fig. 1 Univariate survival analysis for rs662 SNP under the dominant model

Importantly, the association with reduced survival was confirmed by multivariate analysis, using gender, age at onset, and site of onset as covariates (OR = 1.38; 95% CI = 1.07–1.77; $p = 0.012$). Conversely, no association with survival was observed under the recessive and additive models ($p = 0.458$ and $p = 0.054$, respectively). No association with site of onset was observed under the recessive model ($p = 0.39$), while under the additive model there was an association of allele G with bulbar onset ($p = 0.034$), which was confirmed in the comparison between genotypes AA and GA ($p = 0.013$) but not in the comparison between genotypes GA and GG ($p = 0.961$).

Discussion

While previous studies have focused on the comparison between sALS patients and controls with the aim of investigating a possible influence of *PON* gene polymorphisms on susceptibility to sALS, our work is a case-only study on the putative role of *PON1* as modifier gene in ALS. More specifically, our findings suggest that rs662 is associated with bulbar onset and with reduced survival in ALS. Notably, the latter association is independent of site of onset.

Previous studies have inconsistently suggested an effect of *PON* SNPs on ALS susceptibility, without finding an effect on phenotype [8, 13]. Pertaining to rs662, Slowik et al. [8] found an association between the minor allele G and risk of sALS under the additive, dominant, and recessive models, while other studies did not confirm the association [7, 9–12, 14]. Cronin et al. [7] found an association of sALS susceptibility with another coding polymorphism of *PON1*, L55M, and an intronic variant in *PON3* ($PON3_{INS2+3651A>G}$). Landers et al. [9] found an association with sALS for a common *PON1* haplotype comprising five SNPs which did not include rs662. Valdmanis et al. [14] reported an association with a haplotype in *PON2*, while Saeed et al. [11] found sALS to be associated with a haploblock spanning *PON2* and *PON3*. On the other hand, two further studies from Italy and the Netherlands respectively did not find any association between *PON* gene polymorphisms and sALS risk [17, 18]. Finally, of two meta-analyses investigating the association of *PON* SNPs with sALS risk, one reported no association [10], while the other confirmed the association of rs662 with sALS, but the finding did not retain statistical significance after correction for multiple testing [12].

Our findings raise the possibility that certain subpopulations of motor neurons (i.e., bulbar ones) suffer more or earlier from the presence of the minor allele of rs662, and possibly that motor neurons in general undergo faster degeneration if a patient carries this allele. Indeed, rs662 SNP has a functional impact: it determines a glutamine-to-arginine substitution which modifies enzyme activity with regard to substrate specificity and catalytic efficiency [3]. The 192R isoenzyme hydrolyzes more efficiently paraoxon and chlorpyrifos oxon, while the 192Q variant is more efficient with the nerve gases sarin and soman [19–22]. Given that the 192R polymorphism seems to confer higher risk of sALS and, among ALS patients, a higher probability of bulbar onset and a shorter survival, it can be hypothesized that motor neurons are especially vulnerable to those organophosphate compounds which are hydrolyzed less efficiently by the corresponding variant of the enzyme.

However, the elegant study by Wills et al. [13] conducted on 140 ALS patients and 153 controls, though confirming the association between 192R and sALS risk, did not detect any significant differences in the paraoxonase, diazoxonase, or arylesterase activities in serum or cerebrospinal fluid (CSF) of patients and controls. More precisely, there was only a trend toward significance of higher paraxonase activity in the serum of ALS patients, correlating with increased frequency of RR homozigosity in ALS. The authors concluded that the lack of significant difference in these enzymatic activities did not support a role of organophosphate hydrolysis in the influence of Q192R polymorphism on ALS susceptibility. Nevertheless, this cannot be considered as definitive evidence, because in this study enzyme activities were necessarily measured ex vivo and not in vivo, i.e., where the enzyme actually exerts its physiological activities, and hydrolytic activity against only a selected group of organophosphate compounds (i.e., paraoxon (paraoxonase), diazoxon (diazoxonase), and phenylacetate (arylesterase)) was tested, not covering the entire class of these substances (e.g., the organophosphate pesticides parathion, chlorpyrifos, and diazinon, as well as the nerve gases soman and sarin, were not included). These specific compounds could be of relevance in ALS pathogenesis, as suggested by epidemiological studies. On one hand, exposure to pesticides has been consistently linked to increased ALS risk [23, 24]. On the other hand, an increased incidence of ALS has been reported in veterans of the first Gulf War, and it has been hypothesized that this could be attributed to exposure to nerve gases [5, 25]. Interestingly, lower paraoxonase activity was found in the serum of Gulf War veterans developing neurological complaints known as the Gulf War Syndrome, pointing to a putative etiologic role of organophosphate compounds [26, 27].

Apart from its role in hydrolyzing organophosphates, PON1 has also antioxidant properties, as demonstrated by its ability to protect circulating low-density lipoproteins (LDLs) from lipid peroxidation [28]. The 192R isoenzyme binds less efficiently to HDLs and consequently displays reduced stability, reduced lipolactonase activity, and reduced stimulation of cholesterol efflux from macrophages [29]. Accordingly, the R allele confers reduced ability to protect LDL from oxidation [30]. As oxidative stress plays a role in ALS pathogenesis [6], it could be hypothesized that the presence of the 192R isoenzyme increases ALS susceptibility and accelerates disease progression by weakening a systemic protective mechanism against oxidation. This would be in agreement with the finding that serum from ALS patients displays an increase of markers of lipid peroxidation in comparison with healthy controls [31]. As intensive physical exercise is known to increase oxidative stress [32], this mechanism could provide a link between the epidemiological association with physical activity which has been proposed for ALS [33], as well as the increased susceptibility to the disease for certain categories of sports professionals, such as football [34] or American football [35] players.

Our study is the first to suggest a role of a *PON* SNP as disease modifier in ALS, thus strengthening the view that common genetic variants can modify ALS phenotype. Moreover, it can provide support to the hypothesis that certain endogenous damaging processes (i.e., oxidative stress) or exogenous toxic substances (i.e., organophosphate compounds such as pesticides or nerve gases) could play a role in ALS pathogenesis. Further studies, especially laboratory investigations, will be needed in order to further clarify the putative role of rs662 on ALS pathogenesis. For example, in vitro studies on cells carrying the different variants of these polymorphisms (AA, GA, GG) could disclose the different pathophysiological responses of the cells to potentially toxic endogenous or exogenous substances with a possible role in ALS. Particularly informative would be investigations on motor neurons, e.g., as produced through differentiation of induced pluripotent stem cells (iPSCs) obtained from peripheral tissues of patients carrying the different variants of the polymorphism. These laboratory studies could deepen our knowledge of ALS pathogenesis and ultimately lead to therapeutic progress for this currently incurable disease.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and informed consent The study was in accordance with the ethical standards of the institutional research committee. Patients gave their written informed consent to the study.

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