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# Pharmacogenetic interactions in amyotrophic lateral sclerosis: a step closer to a cure?

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## Abstract

Genetic mutations related to amyotrophic lateral sclerosis (ALS) act through distinct pathophysiological pathways, which may lead to varying treatment responses. Here we assess the genetic interaction between *C9orf72*, *UNC13A*, and *MOBP* with creatine and valproic acid treatment in two clinical trials. Genotypic data was available for 309 of the 338 participants (91.4%). The *UNC13A* genotype affected mortality ( $p = 0.012$ ), whereas *C9orf72* repeat-expansion carriers exhibited a faster rate of decline in overall ( $p = 0.051$ ) and bulbar functioning ( $p = 0.005$ ). A dose-response pharmacogenetic interaction was identified between creatine and the A allele of the *MOBP* genotype ( $p = 0.027$ ), suggesting a qualitative interaction in a recessive model (HR 3.96,  $p = 0.015$ ). Not taking genetic information into account may mask evidence of response to treatment or be an unrecognized source of bias. Incorporating genetic data could help investigators to identify critical treatment clues in patients with ALS.

## Introduction

Amyotrophic lateral sclerosis (ALS) is notorious for its genetic, clinical, and etiological diversity [1, 2]. The heterogeneous nature of ALS complicates the design and conduct of clinical trials and may suggest that ALS is not treatable as a single entity; patients may respond differently to treatment and multiple personalized therapies may be required [1, 3, 4].

Although the pathophysiological mechanisms underlying ALS are unclear, it is probable that ALS-related genetic mutations act through multiple, distinct pathways which, in the end, all lead to motor neurodegeneration [5]. A novel

treatment, therefore, may only be effective in patients with a specific mutation, leading to varying treatment responses during clinical trials. As a proof of concept, a recent meta-analysis indicated that the response to lithium depended on the *UNC13A* genotype [2]. This observation points towards a necessity to take genetics into account in future ALS clinical trials, especially if one is interested in genotype-specific treatment effects [6–8].

Despite a handful of genotype-target trials [9–11], the vast majority of ALS trials ignore the potential value of genetic information. In this study, we therefore assess and illustrate how different genotypes may interact within multiple aspects of ALS clinical trials (e.g., baseline balance, efficacy endpoints, and treatment response), based on data of two completed trials. We estimate the effects of three prevalent ALS-related genes (*UNC13A*, *C9orf72*, and *MOBP*), as well as the effect of a less prevalent gene (*SOD1*).

## Materials and methods

### Individual participant data

Data for this study originated from two randomized, double-blind clinical trials conducted in The Netherlands. Both

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trials implemented a fully sequential design, with interim analyses conducted after each pair of events. The first trial aimed to determine the efficacy of creatine monohydrate as compared with placebo on overall survival [12]. A total of 175 patients were enrolled between June 2000 and December 2001 at which point the trial was stopped for futility. The second trial aimed to determine the efficacy of valproic acid (VPA) as compared with placebo on overall survival [13]. Similarly, the trial was stopped for futility having enrolled 163 patients between April 2004 and January 2007.

## Genetic data and genotyping

When conducting post-hoc analyses in clinical trials it is important to recognize that there are three major limitations: (1) lack of within-subgroup randomization, (2) multiplicity of statistical testing and (3) lack of statistical power and precision (i.e., reduced sample size) [14]. These limitations become especially apparent in genetic post-hoc analyses (i.e., pharmacogenetic interaction), which may quickly result in the analysis of dozens of low prevalent genes. We selected, therefore, five candidate genes that were confirmed to be ALS-related and had a minor allele frequency (MAF) of at least 0.15 among Dutch patients: *SCFD1* (rs10139154, MAF 0.331), *SARM1* (rs35714695, MAF 0.162), *UNC13A* (rs12608932, MAF 0.403), *MOBP* (rs616147, MAF 0.302), and the *C9orf72* repeat-expansion [15–17]. The observed variability among the trial participants was, however, too low (i.e., less than ten cases) for *SCFD1* and *SARM1*, and these genes were omitted from the analysis. Genetic variability among trial participants is essential to ensure viable sample sizes within genetic subgroups. Pharmacogenetic analysis based on rare genes, such as *SOD1*, *FUS*, or *TARDBP* (prevalence <1%) [18], were not considered to be feasible, as subgroups would most likely consist of only one or two patients. *UNC13A* and *C9orf72* have been described previously [2]. *MOBP* was recently discovered as an ALS-related gene in a large genome-wide association study [17]. Importantly, the minor allele-frequency of *MOBP* is ~30% in patients with ALS, which would ensure sufficient variability in alleles among trial participants. During the conduct of the VPA and Creatine studies, participants were given the option to provide blood samples for future use. Most blood samples had been used in previous genetic studies (~75%, Fig. 1) and data were readily available [15, 17]. For the remaining samples (~25%), *C9orf72* was genotyped using repeat-primed PCR as described previously [19]. We classified patients with more than 30 repeats in the *C9orf72* gene as *C9orf72* carriers [20]. The *UNC13A* and *MOBP* SNPs were genotyped using Taqman (Applied Biosystems, Foster City, CA) assays as described previously [21].

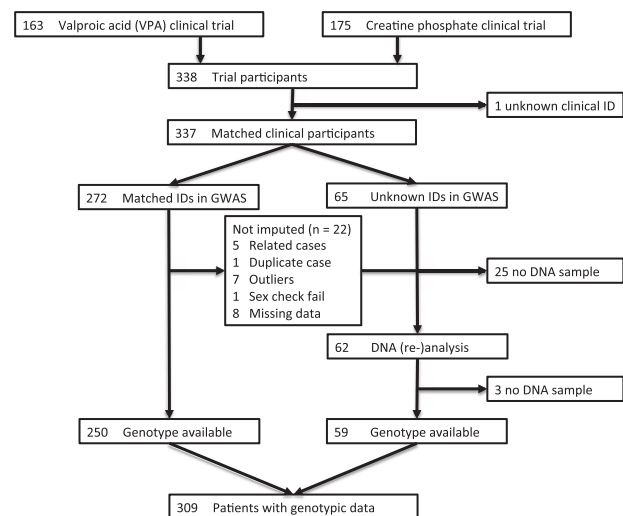
## Standard protocol approvals, registrations, and patient consents

The medical ethics committee and institutional review board of the University Medical Center Utrecht approved this study. All study participants provided written informed consent.

## Statistical analysis

The primary endpoint was mortality, defined as the time from randomization until death from any cause. We used Cox proportional hazards models to analyze the interaction between treatment (placebo vs. intervention) and genotype. To test the significance of each pharmacogenetic interaction we fitted two models: (1) model with treatment and genotype and (2) model with treatment, genotype and the interaction treatment × genotype. A likelihood ratio test was used to compare the two models. All models were adjusted for the ENCALIS survival risk prediction to adjust for group imbalances and to increase the power of the Cox model [22, 23]. Forest plots were used to visually inspect the relationship between genotype and treatment effect.

We hypothesized that, if an allele has a biological interaction with the treatment, the treatment effect alters as a function of the allele. For example, if the treatment effect is associated with the A allele, the treatment effect would be absent in the CC genotype, mediocre in AC, and large in AA. In order to further explore the direction of the interaction, we recoded the genotype to either a dominant (i.e., AA vs. AC+CC) or recessive (i.e., AA+AC vs. CC) model. Qualitative interactions were defined as interactions with opposing treatment effects. Quantitative interactions were



**Fig. 1** Flowchart of genotype matching. For 29 (8.6%) patients, no DNA sample could be matched with the clinical trial data. One participant could not be matched with the hospital information system; the remaining 28 patients never provided a DNA sample

defined as an alternating treatment response across subgroups, but in the same direction [24]. Linear mixed effects models were used for the analysis of the secondary endpoints (ALS functional rating scale [ALSFERS] and %predicted forced vital capacity [FVC]). The more commonly used revised ALSFRS (ALSFRS-R) had not yet been implemented during the Creatine trial; we, therefore, used the ALSFRS in order to harmonize the two trials. All statistical analyses were conducted using the R package *survival* (version 2.42–6, Therneau TM, 2018) and *lme4* (version 1.1–18–1, Bates D, 2018). Due to the explorative nature of this study, results were considered significant when alpha was smaller than 5%.

## Results

In total, 338 unique patients participated in the Creatine (2001) and VPA (2009) studies [12, 13]. Genotypic information regarding the *MOBP*, *C9orf72*, and *UNC13A* genes was available for 309 participants (91.4%); the workflow to match DNA profiles is shown in Fig. 1. 29 cases never provided a DNA sample. Their 12-month survival was found to be worse than participants whose DNA was available: 46% (95% CI 30–70) vs 79% (95% CI 74–84),  $p < 0.001$ . Interestingly, missing DNA was related to treatment allocation; relatively more data were missing from those participants who were receiving active treatment (placebo 4.8% vs active 12.4%,  $p = 0.022$ , Table 1). As all prognostic factors were balanced across treatment arms [12, 13], this observation seems to be related to dropout due to treatment-specific adverse events.

## Genetic distribution at baseline

Table 1 summarizes the distribution of genotypes among treatment arms, stratified by trial. Genotypes were equally distributed in the VPA study. In the Creatine study, however, there were relatively large imbalances (e.g., *UNC13A* AA genotype [26 vs 40%]). In order to put these imbalances into context, we calculated the probability of an imbalance for various sample sizes and prevalences (Table 2) [14]. The risk of a large imbalance ( $\geq 10\%$ ) remains substantial until the total sample size reaches 200 or more for high prevalent genotypes such as *UNC13A* CC or *MOBP* GG. The risk of any meaningful imbalance is negligible for low prevalent genes (1% or less, e.g., *SOD1*). To exemplify, ~1 in 4 trials (23.9%) with a total sample size of 100 risks a  $\geq 10\%$  imbalance in the prevalence of the *UNC13A* CC genotype, whereas this risk is only 0.02% (2 in 10,000 trials) for *SOD1*.

## Interaction genotype and outcome

The *UNC13A* genotype affected mortality during the trial ( $p = 0.012$ ). This effect remained after taking into account the between-trial variability ( $p = 0.048$ ). A dose-response effect of the C-allele was identified in both trials:  $HR_{AA \text{ vs } CA}$  1.6 (95% CI 0.9–2.6) and  $HR_{AA \text{ vs } CC}$  2.1 (95% CI 1.2–4.0, Fig. 2). Interestingly, *C9orf72* did not affect mortality ( $p = 0.77$ ), but repeat expansion carriers did exhibit an accelerated monthly rate of decline in ALSFRS total score as compared with wild type carriers (1.20 [CI: 0.91–1.48] vs 0.90 [CI: 0.81–0.99],  $p = 0.051$ ). This observation was primarily driven by a faster monthly rate of decline in the

**Table 1** Distribution of genes among treatment arms stratified by trial

Gene	Creatine (2001)			Valproic acid (2009)		
	All (N = 175)	Active (n = 88)	Placebo (n = 87)	All (N = 163)	Active (n = 82)	Placebo (n = 81)
<i>C9orf72</i>						
Missing	18 (10%)	13 (15%)	5 (6%)	11 (7%)	8 (10%)	3 (4%)
Normal	146 (93%*)	72 (96%*)	74 (90%*)	138 (91%*)	67 (91%*)	71 (91%*)
Repeat expansion	11 (7%*)	3 (4%*)	8 (10%*)	14 (9%*)	7 (9%*)	7 (9%*)
<i>UNC13A</i>						
Missing	19 (11%)	13 (15%)	6 (7%)	12 (7%)	8 (11%)	4 (7%)
AA	51 (33%*)	21 (26%*)	30 (40%*)	63 (42%*)	34 (39%*)	29 (44%*)
AC	74 (47%*)	41 (51%*)	33 (44%*)	64 (42%*)	30 (46%*)	34 (39%*)
CC	31 (20%*)	19 (23%*)	12 (16%*)	24 (16%*)	13 (15%*)	11 (17%*)
<i>MOBP</i>						
Missing	19 (11%)	13 (15%)	6 (7%)	15 (9%)	9 (10%)	6 (5%)
AA	23 (15%*)	15 (20%*)	8 (10%*)	14 (9%*)	6 (8%*)	8 (11%*)
AG	63 (40%*)	24 (32%*)	39 (48%*)	54 (37%*)	29 (40%*)	25 (33%*)
GG	70 (45%*)	36 (48%*)	34 (42%*)	80 (54%*)	38 (52%*)	42 (56%*)

\*Percentage based on total number of patients for whom genetic data were available

**Table 2** Probability of imbalance after randomization for various genes

Gene	Prevalence	Group size (n)	Risk of imbalance		
			≥5%	≥10%	≥20%
<i>MOBP</i> GG	0.493	25	88.8%	67.2%	20.3%
		50	76.4%	36.8%	5.7%
		100	52.5%	17.9%	0.6%
		250	30.4%	2.8%	<0.01%
<i>UNC13A</i> CC	0.179	25	85.2%	57.7%	9.5%
		50	69.4%	23.9%	1.3%
		100	40.6%	7.9%	0.03%
<i>C9orf72</i> Repeat	0.081	25	78.9%	42.4%	2.0%
		50	57.7%	9.7%	0.06%
		100	24.1%	1.4%	<0.01%
<i>SOD1</i> <sup>a</sup>	0.01	25	35.6%	4.1%	<0.01%
		50	11.7%	0.02%	<0.01%
		100	0.3%	<0.01%	<0.01%
		250	<0.01%	<0.01%	<0.01%

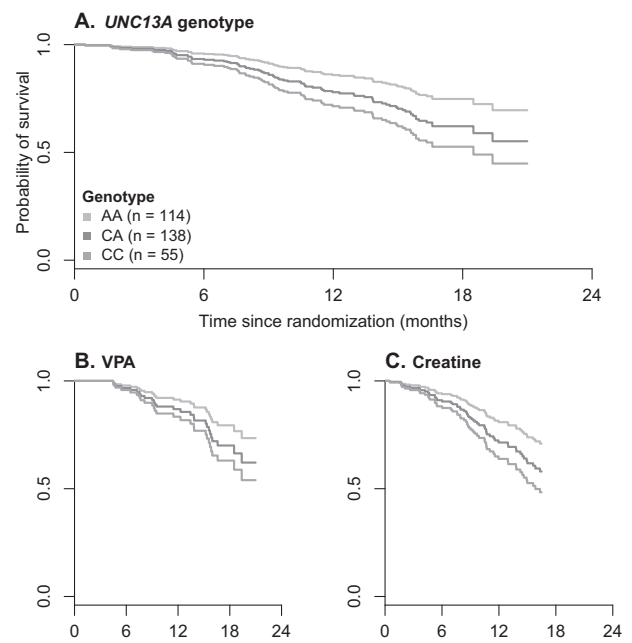
Numbers in the table represent the probability of observing an imbalance between the active and control arm. To exemplify, when the group size is 50 (100 patients in total), the risk that the prevalence of the *UNC13A* CC genotype differs between arms by 10% or more, is 23.9%. The imbalance risk is based on Cui et al. [14], other scenarios can be calculated at <http://reactive.tricals.org>.

<sup>a</sup>SOD1 was not part of the study but included in the table for completeness; prevalence was set to 1% [18, 37]

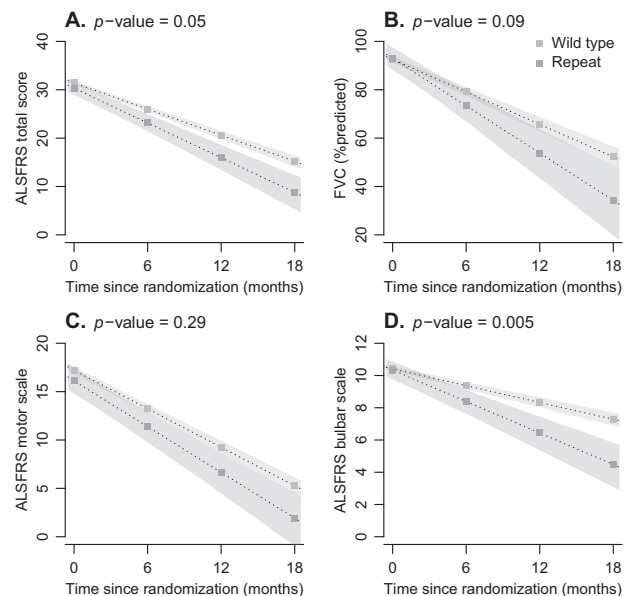
bulbar subdomain (0.32 [CI: 0.22–0.43] vs 0.17 [CI: 0.14–0.22],  $p = 0.005$ , Fig. 3). *UNC13A* did not affect the rate of decline in either ALSFRS total score or FVC ( $p = 0.43$  and  $p = 0.12$ , respectively), although a dose-response relationship was seen in monthly rates of decline in FVC: 2.0% (CI: 1.5–2.6, AA), 2.4% (CI: 1.9–2.8, CA), and 2.7% (CI: 2.0–3.5, CC). *MOBP* did not affect ALSFRS ( $p = 0.35$ ), FVC ( $p = 0.69$ ), or mortality ( $p = 0.38$ ).

### Interaction genotype and treatment

Finally, we evaluated the pharmacogenetic interactions in both studies. In the VPA study, no dose-response patterns were identified and additional analyses were abandoned (Fig. 4a). The overall effect of Creatine on mortality was 0.98 (95% CI 0.60–1.61,  $p = 0.82$ ). Figure 4b reveals a dose-response pharmacogenetic interaction between Creatine and the A allele of the *MOBP* genotype ( $p = 0.027$ ), which was investigated in a recessive model (AA+AG vs GG, HR 3.96 [95% CI 1.27–12.36],  $p = 0.015$ ). This qualitative interaction, with opposing treatment effects, benefited the AA+AG subgroup (HR 0.48, 95% CI 0.22–1.07), but seemed harmful in the GG subgroup (HR 1.90, 95% CI



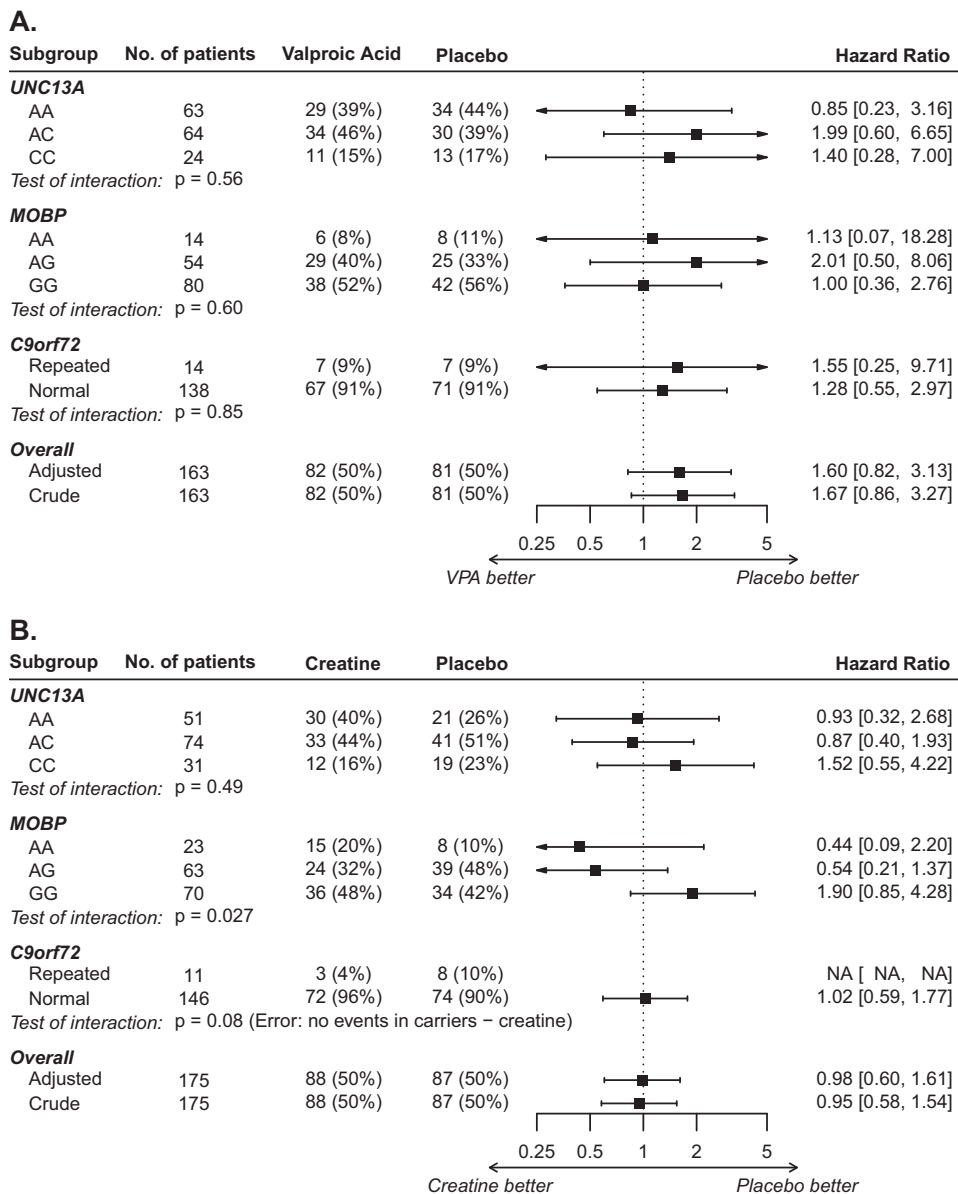
**Fig. 2** Dose-response effect of the *UNC13A* C allele on mortality. The overall (a) and stratified effects of *UNC13A* on mortality in VPA (2009, b) and Creatine (2001, c) studies. Kaplan–Meier curves are based on a Cox proportional hazards model adjusted for the predicted mortality risk [23].



**Fig. 3** Longitudinal interaction between the *C9orf72* genotype with ALSFRS and FVC. **a** ALSFRS total score; **b** FVC %predicted; **c** ALSFRS motor scale (items 4–9); **d** ALSFRS bulbar scale (item 1–3). In red are the *C9orf72* repeat-expansion carriers. Confidence intervals (10th–90th percentile) were bootstrapped ( $n = 1000$ )

0.85–4.28). The interaction between *C9orf72* and Creatine could not be determined due to the small sample size and lack of events within patients with a repeat expansion.

**Fig. 4** Assessment of dose-response pharmacogenetic interactions in the Creatine and VPA studies. Results from Cox models are presented; for each gene we evaluated the interaction with the treatment (**a** VPA; **b** Creatine). We provide the individual hazard ratios per genetic subgroup. Models were adjusted for the ENCALS risk



**Discussion**

In this study, we show how different genotypes interact within multiple aspects of ALS clinical trials. First, the genotype can have a considerable influence on both primary and secondary endpoints. Second, the risk of observing an imbalance at baseline in prevalent genotypes, such as *C9orf72* repeat-expansion and *UNC13A* CC, is substantial. Albeit this finding may not be surprising, it is an important consideration for the development of personalized medicine and is rarely accounted for in current clinical trials. This becomes especially decisive when one assumes the existence of variable treatment responses due to cohort heterogeneity, a hypothesis that is supported by the pharmacogenetic interaction between *MOBP* and creatine.

Not taking genetic information into account may, therefore, mask evidence of response to treatment or be an unrecognized source of bias. The incorporation of genetic data could improve future ALS trials by, for example, adaptively enriching trial populations or reestimating sample sizes.

The relationship between various genes (e.g., *SOD1*, *C9orf72*, and *UNC13A*) and mortality is well established in observational data [23, 25, 26]. Similar associations between *UNC13A* and *C9orf72* with survival were shown in trial participants during a recent meta-analysis [2]. Our results extend these earlier findings by revealing a dose-response relationship with mortality as function of the *UNC13A* C-allele in two independent clinical trials. In addition, we show how *C9orf72* and *UNC13A* may affect the rate of decline in ALSFRS total scores or FVC.

Interestingly, there was a clear effect of *UNC13A* on survival time, but not on ALSFRS. This could be the result of the inflated variability of the ALSFRS [27]. The inflated variability (or dilution) was illustratively shown by the *C9orf72* genotype, where there was a strong bulbar effect ( $p = 0.005$ , consistent with the phenotype) but no motor effect ( $p = 0.29$ ). As a result, the overall, summed, effect of *C9orf72* on ALSFRS total score was only marginally significant ( $p = 0.051$ ). Recognizing the interaction between outcome and genotype is important for future clinical trials, not only in order to reveal potential weaknesses in outcomes, but also to identify potential sources of bias, as well as potential responsive subgroups.

Functional and histopathological studies on the most commonly mutated genes in familial ALS, *SOD1* [28], and *C9orf72* [16, 29], underline the view that different disease mechanisms are at play in ALS. In *C9orf72*-related ALS three different, but not mutually exclusive, mechanisms have been proposed [30]: haplo-insufficiency, RNA toxicity, and the production of presumed toxic dipeptide repeats. Although the aforementioned mechanisms appear to be unique to *C9orf72*-related ALS, cytoplasmic mislocalization, and aggregation of TDP-43, the pathologic hallmark of ALS, are also seen in these cases. In mutant *SOD1* and *FUS*-related ALS, the primary pathology is, however, not TDP-43-related, but rather an aggregation of misfolded mutant protein [31, 32], which may suggest that neurodegeneration is caused through other pathways in this form of ALS. The potential variety in disease mechanisms supports the hypothesis of pharmacogenetic interactions in ALS. Interestingly, we identified a potential heterogeneous treatment response of creatine as function of the A allele of *MOBP*. The biological mechanism remains, however, speculative. *MOBP* may be related to mitochondrial signaling [33], where creatine was believed to attenuate mitochondrial dysfunction [12]. Nevertheless, it could also be that *MOBP* is mere surrogate of a nearby, yet unknown gene [2].

Moreover, as with any post-hoc analysis, this interaction requires external validation before definite conclusions can be drawn [2].

Unfortunately, the larger phase II/III trials are often the first opportunity to evaluate pharmacogenetic interactions in ALS. Our results illustrate the merit of genotypic data for ALS clinical trials, but simultaneously underscore the complexity of these pharmacogenetic interactions. First, there are many ALS-related genes, which may quickly lead to multiplicity issues [14]. Second, ALS-related genes often have a low, geographically depended prevalence, which reduces the available sample size (and power) and could complicate analyses (e.g., lack of events in the *C9orf72*–Creatine interaction, Fig. 4a). Third, retrospective

analyses of the DNA material may lead to an additional selection bias [8, 14], exemplified in our results where patients whose DNA profile was not known, had a worse survival.

Prospectively incorporating the genotype at the design stage could reduce these limitations (e.g., obtaining DNA material at screening, prespecifying subgroup analyses or using stratified randomization), but may not be sufficient. Innovative strategies are, therefore, needed to efficiently detect pharmacogenetic interactions in ALS clinical trials. Incorporating adaptive elements, such as sample size reestimation, or population enrichment, may help to prospectively identify pharmacogenetic interactions [34–36]. If during an interim analysis a differential treatment effect exists, the sample size could be reestimated to improve the analytical precision at trial completion. As alternative, one could opt to only continue with the responding subgroup [34]. These adaptive elements could play a central role in trials for common ALS-related genes such as *UNC13A*, *C9orf72*, and *MOBP* [36]. Nevertheless, when the genetic variation is rare, these methods may fall short and dedicated trials, such as the *SOD1* antisense trial [9], may be our only option.

In conclusion, in this study we assessed the interaction between three common ALS-related genes and two clinical trials. Our results illustrate the value of incorporating genotypic information in ALS clinical trials, but also highlight the challenges for future pharmacogenetic trials. Incorporating genetic data into future ALS clinical trials could improve studies by, for instance, adaptively enriching trial populations or reestimating sample sizes. Ultimately, this strategy could help investigators to identify critical treatment clues in patients with ALS.

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## Compliance with ethical standards

**Conflict of interest** RPAvE, SN, MDJ, H-JW, KRvE, RAAvdS, JJFavV, SP, G-JG, JHV, MJCE report no disclosures. MAvE received grants from the Netherlands Organization for Health Research and Development (Veni scheme), The Thierry Latran foundation and the Netherlands ALS foundation (Stichting ALS Nederland), the EU Joint Programme—Neurodegenerative Disease Research (JPND). LHvdB reports grants from Netherlands ALS Foundation, the Netherlands Organization for Health Research and Development (Vici scheme), the Netherlands Organization for Health Research and Development (SOPHIA, STRENGTH, ALS-CarE project), funded through the EU Joint Programme—Neurodegenerative Disease Research, JPND), served on the Scientific Advisory Board of Biogen, Cytokinetics, Prinses Beatrix SpierFonds, and the Latran Foundation.

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