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PGC-1α is a male-specific disease modifier of human and experimental amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a devastating, adult-onset neurodegenerative disorder of the upper and lower motor systems. It leads to paresis, muscle wasting and inevitably to death, typically within 3–5 years. However, disease onset and survival vary considerably ranging in extreme cases from a few months to several decades. The genetic and environmental factors underlying this variability are of great interest as potential therapeutic targets. In ALS, men are affected more often and have an earlier age of onset than women. This gender difference is recapitulated in transgenic rodent models, but no underlying mechanism has been elucidated. Here we report that SNPs in the brain-specific promoter region of the transcriptional co-activator PGC-1α, a master regulator of metabolism, modulate age of onset and survival in two large and independent ALS populations and this occurs in a strictly male-specific manner. In complementary animal studies, we show that deficiency of full-length (FL) Pgc-1α leads to a significantly earlier age of onset and a borderline shortened survival in male, but not in female ALS-transgenic mice. In the animal model, FL Pgc-1α-loss is associated with reduced mRNA levels of the trophic factor Vegf-A in males, but not in females. In summary, we indentify PGC-1α as a novel and clinically relevant disease modifier of human and experimental ALS and report a sex-dependent effect of PGC-1α in this neurodegenerative disorder.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disorder that affects 2 to 3 per 100 000 people each year with a peak age of onset of 58–63 years (1). Men are more frequently affected than women, resulting in a male to female ratio of 1.4 in Europe (2). Epidemiological studies also show that male patients tend to have an earlier age of onset, a difference recapitulated in selected transgenic rodent models (3). The cause for this gender bias remains enigmatic. Pathologically ALS is characterized by the degeneration of upper and lower motor neurons, which results in paresis, muscle wasting and inevitably in death, typically within 3–5 years, but this can vary in extreme cases from a few months to several decades. Etiologically, ALS is heterogeneous with 5–10% patients reporting a clear Mendelian inheritance hence termed "familial" ALS, while the majority of ALS cases are isolated in nature and are considered 'sporadic' (4). Understanding the genetic and environmental factors that modulate age of onset and disease duration promises to yield important clues for identifying novel therapeutic targets.

PGC-1α, encoded for by the *PPARGC1A* gene, is a transcriptional co-activator that orchestrates the cellular response to metabolic demands (5). Originally described as regulator of mitochondrial respiration in brown adipose tissue, PGC-1α is now known to participate in nearly all cell types in a wide range of ancillary metabolic processes, such as angiogenesis,

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Table 1. *PPARGC1A* rs11737023 and age of onset and age of death in the German ALS cohort

	Rs11737023 G/G	G/A	A/A	*P*-value[a]	*P*-value[b]	*P*-value[c]
Males						
N[d]	219 (105)[d]	118 (64)	16 (5)			
Age of onset	56.0 (47.0–66.0)	59.5 (48.0–66.0)	47.5 (39.0–56.0)	0.0064	0.2104	0.0141
Age of death	64.0 (54.0–69.0)	64.5 (56.0–70.0)	41.0 (34.0–48.0)	0.0973	0.9701	0.0342
Females						
N[d]	133 (79)[d]	86 (44)	18 (11)			
Age of onset	59.0 (52.0–67.0)	58.5 (47.0–67.0)	59.5 (50.0–69.0)	0.9530	0.8778	0.8375
Age of death	66.0 (58.0–71.0)	63.0 (53.5–71.0)	63.0 (59.0–72.0)	0.5815	0.3146	0.9138

Data represent median (lower and upper quartile) in years; statistical significance was calculated using the Kruskal–Wallis test; [a]additive model, [b]dominant model, [c]recessive model; [d]numbers in parentheses indicate number of cases where age of death was available.

antioxidative defense and autophagy (6–9). A recent study revealed that *PPARGC1A* is differentially regulated in the brain versus non-CNS tissues and that variant SNPs in a novel CNS-specific promoter region are associated with a delayed age of symptom onset in Huntington disease (10). In spinal cord and motor cortex tissue from ALS patients and SOD1(G93A) transgenic mice, an animal model of ALS, mRNA and protein levels of PGC-1α are reduced (11). Recently, studies in mouse models of familial ALS demonstrated that transgenic up-regulation of Pgc-1α relieves symptoms and/or extends survival, raising the prospect that this pathway could be targeted therapeutically (12–14). Of note, PGC-1α also regulates a number of pathways protective in ALS, including antioxidant defense (8), and expression of the trophic factor VEGF-A (7). The effects of PGC-1 in human Huntington disease and transgenic models of familial ALS led us to hypothesize that PGC-1α modifies onset and survival in human sporadic ALS in a clinically relevant manner.

RESULTS

PGC-1α as a genetic disease modifier in two independent human ALS populations

To investigate the role of PGC-1α in human ALS, we focused on three SNPs that we used to characterize a role of the brain-specific *PPARGC1A* promoter region in human Huntington disease (10). We thus genotyped DNA from 590 patients (237 females, 353 males) with a clinical diagnosis of sporadic ALS according to the El Escorial criteria (15) and correlated the results with age of onset and—where available—age of death. We find that homozygosity for the minor allele of SNP rs11737023 is associated with an 8-year earlier median age of death in comparison with homozygosity for the major allele (65 versus 53 years). The effect on age of symptom onset is 4 years (57 versus 53 years) but does not attain significance in the analysis of the entire population. Gender stratification revealed that the effects on survival and on symptom onset are confined to the male population of ALS patients, whereas there is no detectable effect of the *PPARGC1A* genotype in women (Table 1).

To control for possible population-based effects, we genotyped rs11737023 in an independent cohort of 464 ALS patients (196 females, 268 males) from Sweden. Disease duration was only calculated in the Swedish population, as age of death and age of onset were only available by year in the Germans. In the confirmation population, we found that, in men, minor allele homozygosity compared with major allele homozygosity for the critical SNP rs11737023 is associated with a 3.3-year earlier median age of death (64.2 versus 67.5 years) and a 50% shorter disease duration (1.5 versus 3.1 years). Once again, however, no effect was observed in the female population (Table 2).

Effect of Pgc-1α deficiency in transgenic mutant SOD1-mediated ALS

To gain insight into the mechanisms underlying the PGC-1α effect in ALS, we turned to a well-characterized transgenic model of mice over-expressing the human ALS-associated *SOD1* G93A mutation (SOD1(G93A) mice) (16). Since up-regulation of Pgc-1α mitigates the disease phenotype in SOD1(G93A) mice, we hypothesized that the disease-accelerating effect in humans might involve a reduced function of PGC-1α. To test this, we crossed SOD1(G93A) mice with Pgc-1α-deficient mice. Two publicly available Pgc-1α −/− mouse strains that differ in several key points were generated by two independent research teams (17,18). Overall, the neurological and metabolic phenotype is less severe in one strain (18), likely due to the knock-out strategy which resulted in a hypomorphic Pgc-1α gene, rather than a complete knock-out as in the other strain (17–19). The gene targeting strategy employed for the hypomorphic model resulted in the duplication of exon 3 that was inserted between exons 5 and 6 and created a coding region frameshift. As a result, a premature termination codon at amino acid 255 was generated that blocks the expression of full-length (FL) Pgc-1α (18). However, a shortened protein termed NT-Pgc-1α^{254} is expressed in the hypomorphic model. Apart from a C-terminal deletion of 16 amino acids, NT-Pgc-1α^{254} is identical to NT-Pgc-1α^{270}, a naturally occurring PGC-1α isoform resulting from alternative splicing (20). In short, the hypomorphic model has a normal life expectancy while displaying a range of milder metabolic abnormalities, such as cold intolerance and mildly deranged body weight regulation (18). Both mouse strains show altered motor activity and a marked vacuolar degeneration of the brain, especially the striatum (18,21). In order to minimize the confounding effects in our behavioral studies, we chose the hypomorphic mouse line (18) for our experiments. In a two-step breeding strategy, we crossed SOD1(G93A) transgenic mice onto a Pgc-1α-deficient C57/B6J background (detailed in Supplementary Material, Fig. S1).

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Table 2. *PPARGC1A* rs11737023 and age of onset, age of death and disease duration in the Swedish ALS cohort

	rs11737023 G/G	G/A	A/A	*P*-value[a]	*P*-value[b]	*P*-value[c]
Males						
N[d]	168 (170)	86 (88)	10 (10)			
Age of onset	63.0 (53.3–70.5)	61.4 (53.2–68.3)	62.4 (59.4–64.0)	0.7287	0.4427	1.0000
Age of death	67.5 (57.8–74.1)	65.1 (57.1–71.2)	64.2 (62.0–65.5)	0.0056	0.0112	0.0099
Disease duration	3.1 (1.9–4.6)	2.4 (1.4–3.8)	1.5 (1.1–2.3)	0.0157	0.0100	0.0530
Females						
N[d]	115 (115)	67 (67)	14 (14)			
Age of onset	66.4 (56.4–71.9)	66.0 (59.5–76.1)	65.3 (58.5–79.0)	0.8992	0.6634	1.0000
Age of death	68.9 (60.7–75.7)	68.6 (61.9–77.9)	67.6 (62.2–80.0)	0.9883	0.8847	1.0000
Disease duration	2.6 (1.7–3.7)	2.2 (1.6–3.4)	3.1 (2.1–4.6)	0.0681	0.1240	0.2587

Data represent median (lower and upper quartile) in years; statistical significance was calculated using the Kruskal–Wallis test; [a]additive model, [b]dominant model, [c]recessive model; [d]numbers in parentheses indicate number of cases where age of death was available.

We then monitored these mice for motor symptoms, body weight and survival (22). We found no significant effect on survival in the gender-mixed SOD1(G93A) cohorts (data not shown). However, gender stratification revealed a borderline significant survival effect of Pgc-1α deficiency in the male cohort, whereas the PGC-1α genotype did not influence the disease phenotype in female mice (Fig. 1A and B). Similarly, Pgc-1α deficiency accelerated disease onset, as defined by the peak of the weight curve of each individual animal (23) in males, while females were unaffected (Fig. 1C and D). Onset of neurological symptoms did not prove to be useful in our system as the Pgc-1α −/− mice showed reduced motor activity independent of the SOD1(G93A) transgene status (data not shown). The recapitulation of the Pgc-1α-linked gender effect in our compound transgenic/knock-out mouse model provided the opportunity for mechanism-based studies.

We determined abundance levels of *Ppargc1a* transcripts initiated at the CNS-specific promoter and the reference gene (RG) promoter in spinal cords of all groups of male and female animals (Supplementary Material, Fig. S2). As expected from our previous studies in mouse brain (10), CNS-specific transcripts were several-fold higher than RG transcripts in the respective groups of male and female mice. No significant sex-specific differences of CNS- and RG-specific transcripts were noted between the respective groups. FL-Pgc-1α deficiency in the SOD1(G93A) mice was associated with the lowest levels of CNS- and RG-specific transcripts in both genders. Interestingly, FL-Pgc-1α deficiency in SOD1(G93A) mice reduced CNS-specific transcripts only in male animals. We also measured transcripts initiated from the alternative exon1 that is specific for the Pgc-1α2 and -α4 isoforms (24). However, the level of these transcripts was more than two orders of magnitudes lower in the spinal cord of male or female control animals than the level of CNS-specific transcripts and such transcripts were below the detection limit of our assay in most animals of the experimental groups (data not shown).

FL-Pgc-1α deficiency is associated with reduced Vegf-A availability in male, but not female ALS mice

ALS is a multifactorial syndrome and a wide range of non-mutually exclusive mechanisms are implicated in its pathogenesis (1). In the context of the present study, dysregulation of the growth factor VEGF-A is of particular interest for three reasons: (i) three different SNPs in the *VEGF-A* gene promoter that are strongly associated with reduced plasma VEGF-A levels are risk factors for sporadic ALS (25) and two reports implicate a gender-dependent effect of the *VEGF*-A SNP status on ALS risk (25,26), (ii) in SOD1(G93A) transgenic rodent models, Vegf-A expression is reduced in the spinal cord and restoration of Vegf-A availability produces a therapeutic benefit (27–30), and (iii) VEGF-A expression is coregulated by PGC-1α via the transcription factor estrogen-related receptor-α (ERRα) (7).

These considerations compelled us to revisit the role of VEGF-A in our model system. As expected and described previously (28,30), *Vegf-A* mRNA levels were reduced in SOD1(G93A) transgenic animals versus controls (Fig. 2A). In males, the Pgc-1α hypomorphic background significantly aggravated the *Vegf-A* deficit, whereas there was no additional effect in females. To ascertain to what extent this reduction is specific to *Vegf-A*, we also measured mRNA levels of a panel of additional neurotrophic factors (*Igf-1*, *Bdnf* and *Ngf*). Whereas *Igf-1* levels were in fact increased in the spinal cords of SOD1-transgenic animals, this effect was significantly attenuated in females on a FL-Pgc-1α-deficient background (Fig. 2B). The other neurotrophic factors were not altered either by SOD1(G93A) expression, FL-Pgc-1α deficiency or both (Fig. 2C and D). The mRNA levels of *Errα* in spinal cord were lower in males than in females of all genotypes except SOD1(G93A) (Supplementary Material, Fig. S3).

DISCUSSION

Here, we present the results of two independent lines of investigation that address the role of PGC-1α in ALS. First, we demonstrate an important disease-modifying role for the PGC-1α encoding gene in patients with ALS. Remarkably, in both European patient populations tested, the effect is strictly confined to men. Second, we show that FL-Pgc-1α deficiency has a similar male-specific, albeit weaker, disease-accelerating effect in the SOD1(G93A) transgenic mouse model of familial ALS. In neither the human nor the animal studies is the mechanism underlying this gender-specific effect immediately evident. However, reduced availability of the neurotrophic factor VEGF-A might be a contributing factor.

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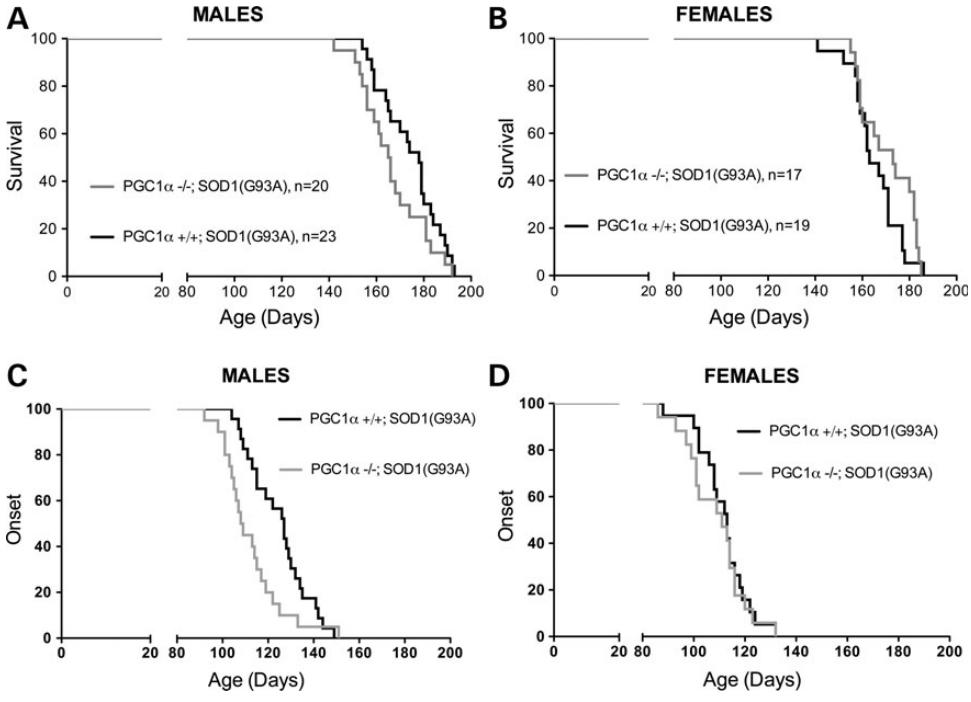

Figure 1. FL-Pgc-1α ablation hastens onset and shortens survival in ALS SOD1(G93A) transgenic mice in a gender-dependent manner. (**A** and **B**) Kaplan–Meier survival plots of SOD1(G93A) transgenic mice on a wild-type or PGC-1α −/− background. (A) Males, median survival 178 days in SOD1(G93A);PGC-1α +/+ mice and 166 days in SOD1(G93A);PGC-1α −/− mice ($P = 0.065$; Wilcoxon test). (B) Females, median survival 163 days in SOD1(G93A);PGC-1α +/+ mice and 173 days in SOD1(G93A); PGC-1α −/− mice ($P = 0.19$; Wilcoxon test). (**C** and **D**) Kaplan–Meier plots of disease onset, defined as the last day of individual maximum weight. (C) Males, median onset 127 days in SOD1(G93A);PGC-1α +/+ mice and 108 days in SOD1(G93A);PGC-1α −/− mice ($P = 0.0014$; Wilcoxon test). (D) Females, median survival 111 days in SOD1(G93A);PGC-1α +/+ mice and 113 days in SOD1(G93A);PGC-1α −/− mice ($P = 0.455$; Wilcoxon test). Group size is the same as indicated in (A) and (B).

Importantly, our two genetic association studies provide the first evidence that PGC-1α, which has already been implicated in Huntington disease and Parkinson disease, also modifies human ALS. We found associations of the PGC-1α-genotype with age of onset in the German population, disease duration in the Swedish population and age of death in both populations. Somewhat unexpected is the finding that in both populations the PGC-1α effect is male specific. However, PGC-1α is known to enhance the transcriptional activities of major sex hormone receptors such as the androgen receptor (31) and the estrogen receptor (32) as well as ERRα (7). Thus a transcriptional effect is plausible since rs11737023 is located in intron 1 near the novel CNS-specific transcription start site. Bioinformatic analyses using a matrix dissimilarity rate of 0 (33) suggest that the three SNPs alter putative transcription factor-binding sites. The C/T substitution at rs2048025 creates a putative binding site (TAAAT) for the pituitary-specific transcription factor POU1F1A (T00691) involved in diverse cellular processes (34). The major A allele at rs17592631 is located in a consensus site (ACAACC/T) for ENGRAILED HOMEOBOX 1 (EN1, T02016) that has been shown to protect dopaminergic neurons from MPTP toxicity in mice (35). This site is altered by the minor T allele. Finally, the substitution of G by A at the discriminatory rs11737023 creates an E-box (CANNTG) that is potentially targeted by multiple basic-loop-helix transcription factors. Specifically, the variant allele sequence CATCTG has been shown to be targeted by NF-X3 (T01514) (36). While NF-X3 has been implicated in B-cell differentiation, putative NF-X3 sites have also been mapped to other genes including the dopamine D2 receptor gene (37).

rs11737023 (or a causative SNP in linkage disequilibrium with it) may alter the CNS-specific PGC-1α expression levels and influence the co-activation of these hormone receptors in a sex-specific manner. However, how such SNP(s) play a role in ALS pathogenesis remains to be investigated. Co-activation of ERRα at the *VEGF-A* promoter by the CNS-specific PGC-1α isoforms may be a potential mechanism. This signaling axis may contribute to the gender effect as we found *Errα* mRNA levels to be lower in male spinal cords compared with females of each phenotype. Furthermore, FL-Pgc-1 deficiency on the SOD1(G93)A background resulted in a significant reduction of CNS-specific *Ppargc1a* transcripts only in male animals. In this context, it is noteworthy that studies of X-linked spinal and bulbar muscular atrophy (SBMA), an adult-onset motor neuron disease, caused by the abnormal expansion of a polyglutamine tract in the androgen receptor, showed that repression of *VEGF-A* led to motor neuron degeneration *in vitro* and *in vivo* in males (38).

The role of PGC-1α in the gender differences of ALS, however, likely is complex and not limited to its effects on VEGF-A and ERRα. For instance, Leone *et al*. (18) described an age-dependent sex-specific obesity in their Pgc-1α hypomorphic mice at 24 weeks which could be protective against ALS (39).

Remarkably, the modifier effect of PGC-1α on ALS onset and survival was much more pronounced in humans than in mice.

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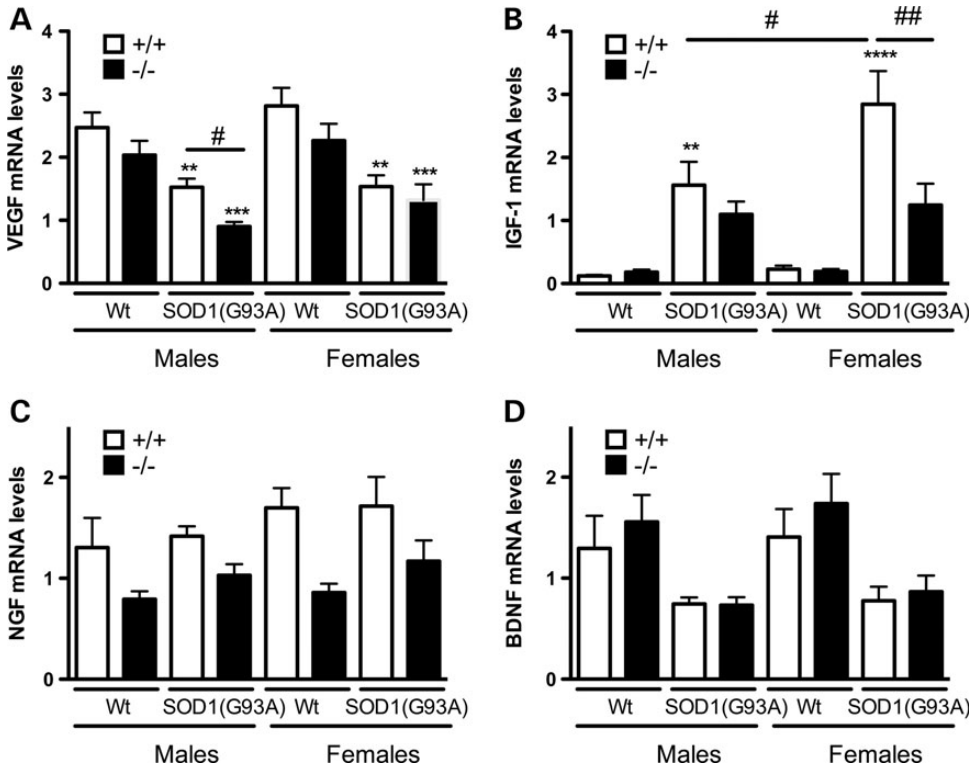

Figure 2. FL-Pgc-1α ablation sex-dependently reduces *Vegf-A* mRNA levels in the spinal cord. (**A**) mRNA levels of *Vegf-A* in the spinal cord of wild-type (+/+) and SOD1(G93A) mice expressing FL-Pgc-1α or not (PGC-1α −/−). $^{*}P < 0.05$ versus Wt, $P < 0.05$ as indicated. Note that *Vegf-A* mRNA levels are decreased in SOD1(G93A) mice and that this decrease is even further reduced in compound male SOD1(G93A)/PGC-1α −/− mice, but not in female SOD1(G93A)/PGC-1α −/−. *wt versus SOD(G93A); #PGC-1α +/+ versus PGC-1α −/−. (**B**) mRNA levels of *Igf-1* in the spinal cord of wild-type (+/+) and SOD1(G93A) mice expressing *FL-Pgc-1α* or not (PGC-1α −/−). $^{*}P < 0.05$ versus Wt; $P < 0.05$ as indicated. $N = 9$ mice per group. *wt versus SOD(G93A); #PGC-1α +/+ versus PGC-1α −/−. (**C**) mRNA levels of *Bdnf* in the spinal cord of wild-type (+/+) and SOD1(G93A) mice expressing FL-Pgc-1α or not (PGC-1α −/−). $^{*}P < 0.05$ versus Wt, $P < 0.05$ as indicated. (**D**) mRNA levels of *Ngf* in the spinal cord of wild-type (+/+) and SOD1(G93A) mice expressing FL-Pgc-1α or not (PGC-1a −/−). $^{*}P < 0.05$ versus Wt; $P < 0.05$ as indicated. For all panels: $N = 9$ mice per group. Statistics were done with one-way ANOVA followed by Newman–Keuls multiple comparison test where appropriate $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.005$. Columns (error bars) represent means (SEM).

This disconnect between human and mouse studies is similar to what has been reported for other modifiers of ALS, namely ephrin 4 (40), and further challenges the sensitivity of SOD1(G93A) transgenic mice as screening tools for ALS therapy research. Along the same lines, the gender effect on survival and disease onset in the SOD1 mouse model is variable and depends, among other factors, on the genetic back ground (3).

In contrast, the mouse studies do offer some mechanistic insight, as they suggest that a sex-dependent reduced availability of Vegf-A in the spinal cord is part of the pathogenic cascade. The increase in *Igf-1* mRNA levels in the spinal cord from SOD1(G93A) mice has been described previously (41). Our observation that this increase is attenuated in the FL-Pgc-1α-deficient mice points to the possible involvement of PGC-1α4, a newly characterized PGC-1α-isoform (24). PGC-1α4, which is closely related to NT-PGC-1α (42), is shorter than the canonical PGC-1α and regulates a gene set somewhat different from the canonical PGC-1α (24). Importantly, PGC-1α4, which is expressed in skeletal muscle, heart and brain, specifically induces IGF-1 (24). We have previously identified a CNS-specific isoform that differs from PGC-1α4 and NT-PGC-1α only by a few amino acids at the N-terminus (10). Even though FL-PGC-1α, PGC-1α4 or NT-PCG-1α is not produced in the hypomorphic mouse model, NT-PGC-1α^{254} is present in these mice. Furthermore, the latter form, initiated at the CNS-specific promoter, is likely to be formed in the FL-Pgc-1α-deficient mouse model. The ability of NT-PGC-1^{254} to interact and co-activate ERRα appeared to be somewhat lower in comparison to the ability of native NT-PGC-1α (42). However, the functionality of such an isoform that is initiated at the CNS promoter needs to be determined.

Notably, rs11737023 is part of a haplotype block with a significant disease modulatory effect in Huntington disease, like SBMA, a polyglutamine disease (10). This observation supports the concept that the pathomechanisms in ALS and Huntington disease are related and, therefore, are of importance for neurodegeneration in general. Nonetheless, it should be emphasized that there are also intriguing contrasts between the PGC-1α effects in the two diseases. Most importantly, the PGC-1α SNP effect tends to be protective in Huntington disease, whereas in ALS it is clearly deleterious. We conclude that while the PGC-1α signaling hub is important in the pathogenesis of both Huntington disease and ALS, its effects are likely mediated by distinct mechanisms that await further studies.

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MATERIALS AND METHODS

Clinical resource

All patients were assessed by experienced clinicians and provided written informed consent. ALS diagnosis was made according to the El Escorial ALS diagnostic criteria (15). The patients were clinically diagnosed and followed at the ALS centers at the Ulm University Hospital and the Charité Hospital (German cohort) and throughout Scandinavia (Swedish cohort). The patients received optimal care according to state of knowledge at the time. For the German population, age of onset and age of death were available only by year, not by exact date. In the Swedish population, exact dates for age of onset (within 2 weeks) were available. To avoid confounders by imprecision, we calculated the disease duration only in the Swedish population. DNA was isolated from whole blood. Extracted DNA was stored at −20°C until analysis. For genotyping of rs11737023, rs2048025 and rs17592631, we used the TaqMan genotyping assays C_473389_10, C_1222369_10 and C-3280847_10.

Animal breeding and genotyping

Pgc-1α −/−; SOD1(G93A) mice were generated using a two-step breeding procedure. In a first step, B6.Cg-Tg(SOD(G93A)) males (stock# 004435, the Jackson laboratory) were bred to B6-Pgc-1α −/− females. In a second step, Pgc-1α +/−, SOD(G93A) males were mated with Pgc-1α +/− female mice (18). Experiments were conducted in double mutant Pgc-1α −/−; SOD1(G93A) and Pgc-1α −/− mice; SOD1(G93A), and wild-type littermates served as controls. Offspring was separated from their mothers at the age of 21 days, followed by labeling and genotyping.

Animals and genotyping

For genotyping of SOD1(G93A) mice, a multiplex-PCR reaction was applied using the following primers. mIL2 forward (CTAGGCCACAGAATTGAAAGATCT) and mIL2 reverse (GTAGGTGGAAATTCTAGCATCATC); hSOD forward (CATCAGCCCTAATCCATCTGA) and hSOD reverse (CGC GACTAACAATCAAAGTGA). Genotyping of Pgc-1α −/− mice was performed according to the protocol published by Leone *et al*. (18).

Acclimatization and housing conditions

At the age of 38 days, mice were moved to the experimental unit and randomly distributed to experimental and control groups. Gender was distributed equally across the groups, with $n =$ 17–23 mice per group. Mice were kept single caged. There was automatic control of light cycle, temperature and humidity. Light hours were 6:00 a.m.–6:00 p.m. Daily monitoring indicated that temperature and humidity remained within the target ranges of 20°C ± 3°C and 80 ± 10%. All experiments were conducted according to the protocol approved by the Regional Steering Committee Tübingen, Reg. 1013.

Motor activity

To monitor activity levels of each animal individually, mice were caged separately with free access to a running wheel from 42 days on. Motor activity is recorded automatically during the nocturnal phase from 6:00 p.m.–6:00 a.m. Motor activity is directly correlated with the rotations per minute generated by each animal on the running wheel. Each full turn of the wheel generates two electromagnetic signals, which are fed directly into an electronic device and saved on a computer. For this purpose, the software 'Mausvital' supplied by Laser und Medizin GmbH, Berlin, was used.

Body weight

Mice were weighed twice weekly starting at the age of 42 days using a digital scale and their weights recorded.

Survival

Pgc-1α −/−; SOD(G93A) and SOD(G93A) mice were sacrificed at the time when they reached the final stage of the disease. As mandated by the Animal Ethics Committee of the Regional Steering Committee Tübingen, the final disease stage is defined as the inability to rise immediately after being placed on the side.

RT-qPCR

Total RNA was extracted using RNeasy Mini Kit (Qiagen) in accordance with the manufacturer's instructions. Real-time RT quantitative PCR was performed with one microgram of total RNA as described (43). PCR analysis was performed on a Bio-Rad iCycler System using iQSYBR Green Supermix. A specific standard curve was performed in parallel for each gene to assess the specificity of the products, for quantification of the respective transcripts in duplicate. PCR conditions were 3 min at 94°C, followed by 40 cycles of 45 s at 94°C and 10 s at 60°C. The relative levels of each RNA were normalized to two housekeeping genes (polymerase II and TBP). CNS- and RG-specific *Ppargc1a* transcripts were quantified using primers targeting CNS-specific exons B1 and B4 (10) and exons 1 and 2, respectively, as described (44). Oligonucleotide sequences are reported in Supplementary Material, Table S1.

Statistics

For the clinical data, allele frequencies were estimated by gene counting. Genotypes associated with the three SNPs typed fulfilled Hardy–Weinberg expectations, as ascertained using a χ^2 goodness-of-fit test. Effects of genotypes on age of onset and age of death, and disease duration were ascertained by the Kruskal–Wallis test rather than ANOVA, inasmuch as the distributions of the respective ages or log-transformed ages significantly deviated from a normal distribution.

For the experimental data, all statistical analysis was done using Prism, version 4.0 (GraphPad Software). For comparison of groups, ANOVA was used. Differences between means

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were determined by *post hoc* comparisons using the Tukey honest significance test and were considered statistically significant if $P < 0.05$. Survival analysis was performed using the Gehan–Breslov–Wilcoxon test.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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