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ORIGINAL ARTICLE

Mutation-specific metabolic profiles in presymptomatic amyotrophic lateral sclerosis

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Abstract

Background and purpose: Growing evidence shows that ALS patients feature a disturbed energy metabolism. However, these features have rarely been investigated in the presymptomatic stage.

Methods: A total of 60 presymptomatic ALS mutation carriers and 70 age- and gender-matched controls (non-mutation carriers from the same families) were recruited. All subjects underwent assessments of their metabolic profiles under fasting conditions at enrollment, including body mass index (BMI), blood pressure and serum levels of blood glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein.

Results: All mutations combined, no differences between presymptomatic ALS gene carriers and controls were found. From a cardiovascular point of view, presymptomatic chromosome 9 open reading frame 72 (*C9ORF72*) gene carriers showed lower cardiovascular risk profiles compared to healthy controls, including lower BMI (median 22.9, interquartile range [IQR] 20.6–26.1 kg/m^2 vs. 24.9, IQR 22.7–30.5 kg/m^2; $p = 0.007$), lower systolic blood pressure (120, IQR 110–130 mmHg vs. 128, IQR 120–140 mmHg; $p = 0.02$), lower fasting serum glucose (89.0, IQR 85.0–97.0 mg/dl vs. 96.0, IQR 89.3–102.0 mg/dl; $p = 0.005$) and higher HDL (1.6, IQR 1.3–1.8 mmol/l vs. 1.2, IQR 1.0–1.4 mmol/l; $p = 0.04$). However, presymptomatic superoxide dismutase 1 (*SOD1*) gene mutation carriers showed higher cardiovascular risk profiles compared to healthy controls, including higher BMI (28.0, IQR 26.1–31.5 kg/m^2 vs. 24.9, IQR 22.7–30.5 kg/m^2; $p = 0.02$), higher fasting serum glucose (100.0, IQR 94.0–117.0 mg/dl vs. 96.0, IQR 89.3–102.0 mg/dl; $p = 0.04$) and lower HDL (1.2, IQR 1.0–1.4 mmol/l vs. 1.4, IQR 1.2–1.7 mmol/l; $p = 0.01$). These features were most prominent in patients carrying *SOD1* gene mutations associated with slow disease progression.

Conclusions: This study identified distinct metabolic profiles in presymptomatic ALS gene carriers, which might be associated with disease progression in the symptomatic phase.

KEYWORDS
amyotrophic lateral sclerosis, genetics, metabolism, presymptomatic gene carriers

Dongsheng Fan, Albert C. Ludolph and Johannes Dorst have contributed equally and they are co-corresponding authors.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive loss of motor neurons leading to muscle atrophy and paresis. The involvement of respiratory muscles causes respiratory insufficiency and death after a mean survival time of only 3–5 years [1]. Despite decades of intensive research, there is still a lack of promising therapeutic strategies. The pathomechanisms behind the disease are still largely unknown. Most ALS patients have a sporadic disease, while approximately 10% of ALS patients have a positive family history (familial ALS, fALS) [2]. A repeat expansion in the chromosome 9 open reading frame 72 (*C9ORF72*) gene and various mutations in the superoxide dismutase 1 (*SOD1*) gene were identified as the most common causes for European fALS, accounting for ~60% of cases [3].

Amyotrophic lateral sclerosis patients feature distinct disturbances of energy [4], glucose [5, 6] and lipid metabolism [7, 8]. They display an increased resting energy expenditure [9, 10], catabolism and weight loss, which usually occurs early during the course of disease [11, 12], even before disease onset [13], and negatively affects prognosis [14, 15]. In the context of these metabolic changes, a mitochondrial [16, 17] and/or hypothalamic dysfunction [18, 19] have been discussed. These findings have prompted clinical trials with high-caloric and/or high-fat nutritional interventions which indicated that such interventions might beneficially influence the course of disease [20–22].

Furthermore, various studies have investigated the association between distinct metabolic profiles and the risk of developing ALS, generally indicating that ALS was associated with lower cardiovascular risk profiles [23–25]. However, these studies refer to the clinical stage of ALS, whilst the metabolic profiles of the presymptomatic stage are largely unknown. Focusing on the presymptomatic stage of ALS may provide a better understanding of the causative interactions between the observed phenomena and potentially highlight novel presymptomatic biomarkers and therapeutic targets.

Therefore, a study was performed in 60 presymptomatic ALS mutation carriers including *C9ORF72* gene, *SOD1* gene, *FUS*, *KIF5A*, *NEK1*, *SETX*, *TBK1* and *TDP43* mutations, and 70 age- and gender-matched controls (non-mutation carriers from the same families) to compare their metabolic profiles, aiming (1) to identify whether ALS patients displayed changes with regard to metabolic profiles in the presymptomatic stage and (2) to investigate whether specific ALS-causing gene mutations were associated with distinct metabolic profiles.

METHODS

Study population

A total of 130 subjects were monocentrically recruited in the Department of Neurology, University of Ulm, Germany, between 2012 and 2021. All subjects provided written informed consent. The Ethics Committee of Ulm University approved the study (approval number 20/12). Presymptomatic ALS mutation carriers ($n = 60$) were recruited from families with a positive history of ALS and a known ALS-causing mutation by genetic testing. All controls ($n = 70$) were first- or second-degree relatives (i.e., parents, children or siblings) of patients with ALS who did not carry pathological mutations.

Assessments of metabolic profiles

Amyotrophic lateral sclerosis mutation carriers and controls underwent the same standardized assessments for metabolic factors at enrollment. Body mass index (BMI) was calculated from weight and height according to the formula BMI = weight in kg/(height in m)^2. Systolic blood pressure (SBP; mmHg) and diastolic blood pressure (DBP; mmHg) were determined manually according to Riva-Rocci. Fasting serum samples were taken for measurement of glucose (mg/dl) and lipids (mmol/l), including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

Statistical analysis

For descriptive statistics, median and interquartile range (IQR) were used. Group comparisons for continuous variables were performed using the two-sample *t* test or the Wilcoxon rank-sum test as appropriate. Two-sided 95% confidence intervals were calculated for mean or median group differences. Group comparisons for categorical variables were performed using a chi-squared test or Fisher's exact test as appropriate. Two-sided 95% confidence intervals were calculated for group differences of proportions. For analyses of *SOD1* gene mutations, normally distributed variables were adjusted for age via a linear regression model. A p value <0.05 (two-sided) was considered as statistically significant. The sample size was determined based on the number of presymptomatic gene carriers and respective controls collected in a monocentric setting within the given time frame. All analyses were performed using the statistical software packages SPSS version 26 (SPSS, Chicago, IL, USA) and GraphPad Prism version 9.1.1 (GraphPad, San Diego, CA, USA).

RESULTS

Between 2012 and 2021, 60 presymptomatic ALS gene mutation carriers and 70 controls (i.e., subjects from the same families as the ALS gene carriers without a disease-causing mutation) were included. Amongst the 60 mutation carriers, 28 were *C9ORF72* gene repeat expansion carriers, 21 were *SOD1* gene mutation carriers and the rest were carriers of various other mutations (Figure 1a). Demographic and clinical characteristics of each group are displayed in Table 1.

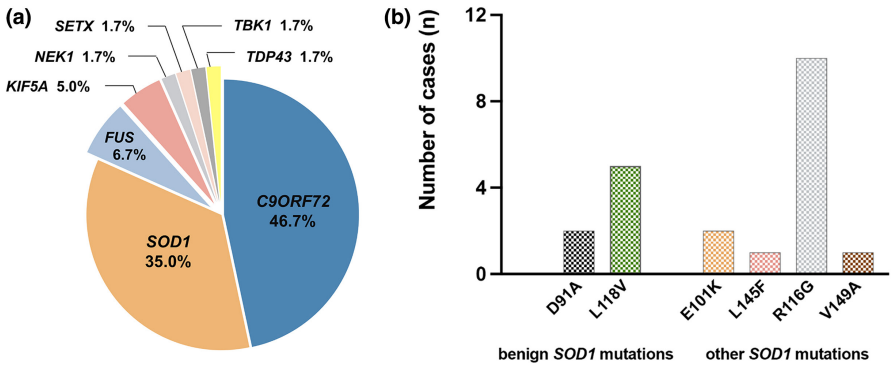

FIGURE 1 Distribution of mutations. (a) Distribution of mutations in the group of ALS gene carriers ($n = 60$). (b) Mutational spectrum of the *SOD1* gene subgroup ($n = 21$). Columns represent absolute numbers. *C9ORF72* gene, chromosome 9 open reading frame 72; *FUS*, fused in sarcoma; *NEK1*, NIMA related kinase 1; *KIF5A*, kinesin family member 5A; *SETX*, senataxin; *SOD1* gene, superoxide dismutase 1; *TBK1*, serine/threonine protein kinase; *TDP43*, transactive response DNA binding protein 43.

TABLE 1 Demographic and clinical characteristics of presymptomatic ALS gene carriers and controls from the same families without respective mutations

	ALS gene carriers (total) ($n = 60$)	*C9ORF72* gene carriers ($n = 28$)	*SOD1* gene carriers ($n = 21$)	Controls ($n = 70$)
Age[a] (years)	45 (32–54)	44 (32–51)	47 (36–65)	43 (30–52)
	$p = 0.44$	$p = 0.95$	$p = 0.10$	
Sex (male, %)	25 (42%)	7 (25%)	14 (67%)	32 (46%)
	$p = 0.64$	$p = 0.06$	$p = 0.09$	
BMI (kg/m^2)	26.1 (22.5–29.0)	22.9 (20.6–26.1)	28.0 (26.1–31.5)	24.9 (22.7–30.5)
	$p = 0.91$	$p = 0.007$	$p = 0.02$	
Fasting serum glucose (mg/dl)	94.0 (87.0–102.0)	89.0 (85.0–97.0)	100.0 (94.0–117.0)	96.0 (89.3–102.0)
	$p = 0.44$	$p = 0.005$	$p = 0.04$	
SBP (mmHg)	120 (112–135)	120 (110–130)	130 (120–140)	128 (120–140)
	$p = 0.28$	$p = 0.02$	$p = 0.73$	
DBP (mmHg)	80 (75–85)	80 (70–80)	80 (75–89)	80 (70–90)
	$p = 0.94$	$p = 0.18$	$p = 0.79$	
TG (mmol/l)	1.1 (0.7–1.7)	1.0 (0.6–1.3)	1.6 (0.9–1.9)	1.0 (0.8–1.5)
	$p = 0.64$	$p = 0.42$	$p = 0.16$	
TC[a] (mmol/l)	5.0 (4.3–6.5)	5.2 (4.6–6.9)	5.0 (3.8–6.3)	5.1 (4.5–5.8)
	$p = 0.79$	$p = 0.44$	$p = 0.61$	
HDL[a] (mmol/l)	1.3 (1.1–1.7)	1.6 (1.3–1.8)	1.2 (1.0–1.4)	1.4 (1.2–1.7)
	$p = 0.42$	$p = 0.04$	$p = 0.01$	
LDL[a] (mmol/l)	3.2 (2.5–4.6)	3.5 (2.8–4.6)	3.2 (2.0–4.6)	3.3 (2.8–4.1)
	$p = 0.89$	$p = 0.50$	$p = 0.56$	

Note: Data are *n* (%) or median (IQR). *p*-values refer to comparisons between respective gene carriers and controls.

Abbreviations: ALS, amyotrophic lateral sclerosis; BMI, body mass index; *C9ORF72* gene, chromosome 9 open reading frame 72; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; *SOD1* gene, superoxide dismutase 1; TC, total cholesterol; TG, triglycerides.

[a]Normally distributed variables.

Age and sex were equally distributed between presymptomatic ALS gene carriers and controls. Considering all mutations combined, there were no differences between mutation carriers and controls for all investigated metabolic factors, including BMI, fasting serum glucose, SBP, DBP, TG, TC, HDL and LDL (Table 1). However, distinct profiles were present for the subgroups of presymptomatic *C9ORF72* gene and *SOD1* gene mutation carriers.

Compared with controls, *C9ORF72* gene mutation carriers had a lower BMI (22.9, IQR 20.6–26.1 kg/m^2 vs. 24.9, IQR 22.7–30.5 kg/m^2; $p = 0.007$), lower fasting serum glucose (89.0, IQR 85.0–97.0 mg/

dl vs. 96.0, IQR 89.3–102.0 mg/dl; $p = 0.005$), lower SBP (120, IQR 110–130 mmHg vs. 128, IQR 120–140 mmHg; $p = 0.02$) and higher HDL (1.6, IQR 1.3–1.8 mmol/l vs. 1.4, IQR 1.2–1.7 mmol/l; $p = 0.04$), corresponding to a lower cardiovascular risk profile (Table 1 and Figure 2a–h). In order to provide higher homogeneity between groups and limit the potential effects of genetic and environmental confounders, also the 28 *C9ORF72* gene-positive presymptomatic gene carriers were compared with 13 *C9ORF72* gene-negative controls from the same families only. This analysis confirmed the same trend for all parameters with significant differences observed when comparing *C9ORF72* gene carriers with all controls; however, due to lower numbers in this subanalysis, only HDL remained statistically significant (1.6, IQR 1.3–1.8 mmol/l vs. 1.2, IQR 1.1–1.3 mmol/l; $p = 0.004$) (Table 2).

On the other hand, compared to controls, *SOD1* gene mutation carriers featured higher BMI (28.0, IQR 26.1–31.5 kg/m^2 vs. 24.9, IQR 22.7–30.5 kg/m^2; $p = 0.02$), higher fasting serum glucose (100.0, IQR 94.0–117.0 mg/dl vs. 96.0, IQR 89.3–102.0 mg/dl; $p = 0.04$) and lower HDL (1.2, IQR 1.0–1.4 mmol/l vs. 1.4, IQR 1.2–1.7 mmol/l; $p = 0.01$) (Table 1, Figure 2a–h). The intrafamily comparison between 21 *SOD1* gene mutation carriers and 11 *SOD1* gene-negative controls from the same families, despite lower numbers, confirmed that carriers had a significantly higher BMI (28.0, IQR 26.1–31.5 kg/m^2 vs. 24.2, IQR 22.1–25.6 kg/m^2; $p = 0.007$) and lower HDL (1.2, IQR 1.0–1.4 mmol/l vs. 1.5, IQR 1.2–1.7 mmol/l; $p = 0.04$); however, in this subanalysis no difference in fasting serum glucose levels was observed (Table 3).

Due to the clinical heterogeneity of different *SOD1* gene mutations and the relatively benign prognosis of the D91A and L118V mutations, carriers of D91A and L118V mutations were also compared with other *SOD1* gene mutation carriers. Out of the 21 *SOD1* gene -positive individuals, seven were carriers of D91A and L118V, consisting of the benign *SOD1* gene mutation group. The complete mutational spectrum of the presymptomatic *SOD1* gene carrier subgroup is given in Figure 1b. It was found that the subgroup with benign *SOD1* gene mutations featured higher fasting serum glucose (111.5, IQR 106.7–116.4 mg/dl vs. 102.1, IQR 95.3–108.9 mg/dl; $p = 0.048$), lower TC (4.2, IQR 3.9–4.5 mmol/l vs. 5.6, IQR 5.3–5.9 mmol/l; $p < 0.001$), lower HDL (1.0, IQR 0.9–1.1 mmol/l vs. 1.3, IQR 1.3–1.4 mmol/l; $p < 0.001$) and lower LDL (2.7, IQR 2.3–3.0 mmol/l vs. 3.8, IQR 3.5–4.0 mmol/l; $p < 0.001$) levels compared to the other *SOD1* gene mutations combined (Table 4, Figure 3a–h).

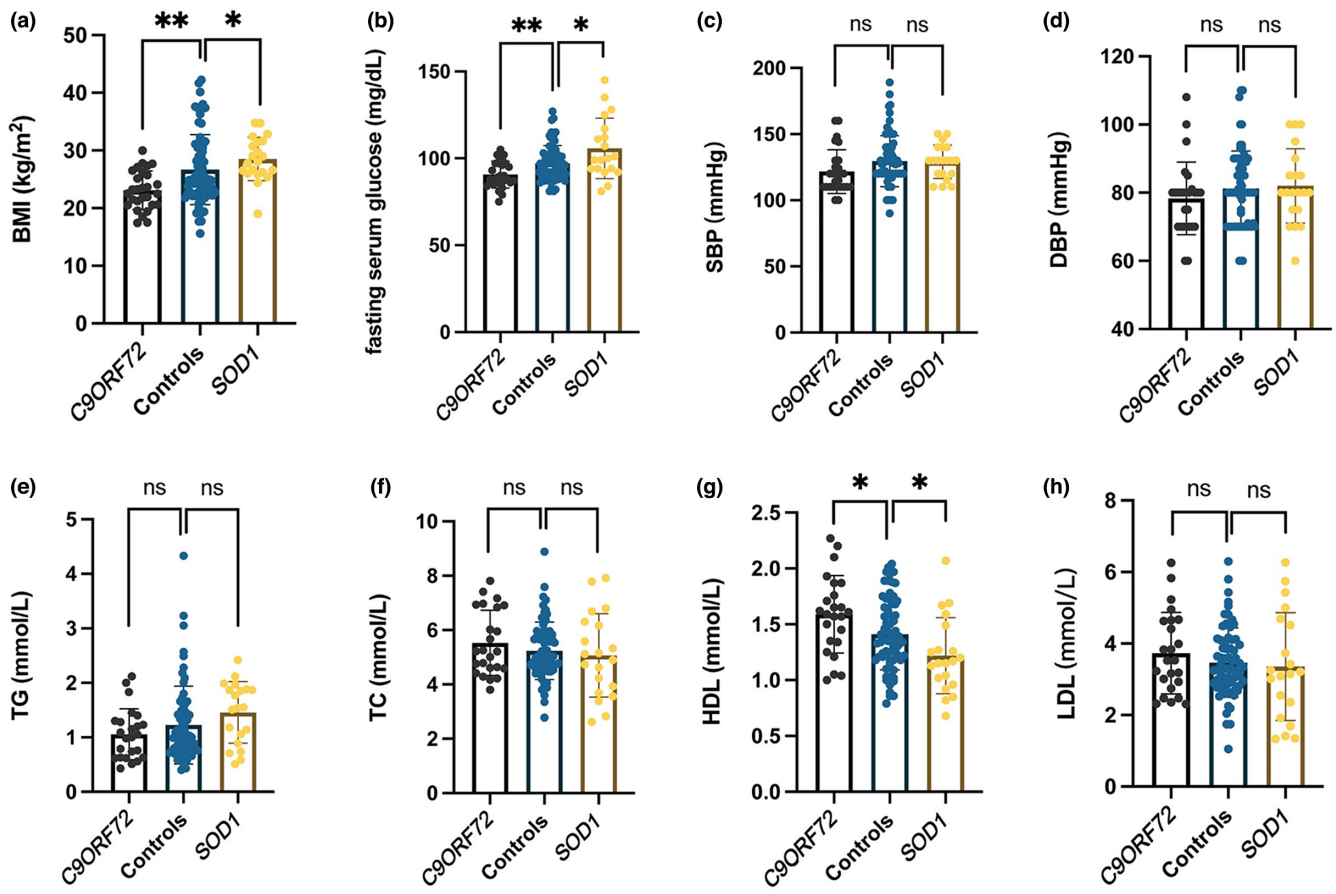

FIGURE 2 Metabolic factors in specific mutations. Plots show median, 95% confidence interval and individual values of all analyzed cardiovascular risk factors for *C9ORF72* gene mutation carriers, *SOD1* gene mutation carriers and controls. Asterisks mark significant (*$p < 0.05$) and highly significant (**$p < 0.01$) differences. ALS, amyotrophic lateral sclerosis; BMI, body mass index; *C9ORF72* gene, chromosome 9 open reading frame 72; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; *SOD1* gene, superoxide dismutase 1; TC, total cholesterol; TG, triglycerides.

TABLE 2 Demographic and clinical characteristics of presymptomatic *C9ORF72* gene carriers and intrafamily controls without respective mutations

	C9ORF72 gene carriers ($n = 28$)	Controls from the same families ($n = 13$)	*p*-value
Age[a] (years)	44 (32–51)	43 (37–55)	0.72
Sex (male, %)	7 (25%)	7 (54%)	0.09
BMI[a] (kg/m^2)	22.9 (20.6–26.1)	25.6 (22.9–33.4)	0.05
Fasting serum glucose[a] (mg/dl)	89.0 (85.0–97.0)	94.5 (87.3–104.8)	0.32
SBP (mmHg)	120 (110–130)	130 (120–140)	0.29
DBP (mmHg)	80 (70–80)	80 (73–90)	0.47
TG[a] (mmol/l)	1.0 (0.6–1.3)	1.2 (0.9–1.8)	0.09
TC[a] (mmol/l)	5.2 (4.6–6.9)	5.7 (4.7–7.0)	0.42
HDL[a] (mmol/l)	1.6 (1.3–1.8)	1.2 (1.1–1.3)	0.004
LDL[a] (mmol/l)	3.5 (2.8–4.6)	3.9 (3.3–5.1)	0.17

Note: Data are *n* (%) or median (IQR).

Abbreviations: ALS, amyotrophic lateral sclerosis; BMI, body mass index; *C9ORF72* gene, chromosome 9 open reading frame 72; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

[a]Normally distributed variables.

TABLE 3 Demographic and clinical characteristics of presymptomatic *SOD1* gene carriers and intrafamily controls without respective mutations

	SOD1 gene carriers ($n = 21$)	Controls from the same families ($n = 11$)	*p*-value
Age[a] (years)	47 (36–65)	36 (28–54)	0.16
Sex (male, %)	14 (67%)	5 (46%)	0.28
BMI[a] (kg/m^2)	28.0 (26.1–31.5)	24.2 (22.1–25.6)	0.007
Fasting serum glucose (mg/dl)	100.0 (94.0–117.0)	99.0 (91.8–103.3)	0.37
SBP (mmHg)	130 (120–140)	130 (120–133)	0.95
DBP[a] (mmHg)	80 (75–89)	80 (80–88)	0.83
TG[a] (mmol/l)	1.6 (0.9–1.9)	1.0 (0.8–1.7)	0.32
TC[a] (mmol/l)	5.0 (3.8–6.3)	5.5 (4.9–5.9)	0.28
HDL[a] (mmol/l)	1.2 (1.0–1.4)	1.5 (1.2–1.7)	0.04
LDL[a] (mmol/l)	3.2 (2.0–4.6)	3.9 (3.3–4.2)	0.27

Note: Data are *n* (%) or median (IQR).

Abbreviations: ALS, amyotrophic lateral sclerosis; BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; *SOD1* gene, superoxide dismutase 1; TC, total cholesterol; TG, triglycerides.

[a]Normally distributed variables.

In addition, the age-adjusted metabolic profiles were compared between benign *SOD1* gene mutation carriers and healthy controls and it was found that the group with benign *SOD1* gene mutations featured lower TC (4.2, IQR 3.9–4.5 mmol/l vs. 5.2, IQR 5.0–5.5 mmol/l; $p < 0.001$), lower HDL (1.0, IQR 0.9–1.1 mmol/l vs. 1.4, IQR 1.4–1.4 mmol/l; $p < 0.001$) and lower LDL (2.7, IQR 2.3–3.0 mmol/l vs. 3.5, IQR 3.2–3.7 mmol/l; $p < 0.001$) levels.

DISCUSSION

In this study, metabolic profiles of 60 presymptomatic ALS gene carriers and 70 age- and gender-matched controls without pathological mutations were compared.

Whereas the combined analysis including all ALS mutation carriers did not reveal significant differences from controls, the mutation-specific analysis showed that *C9ORF72* gene carriers have a lower cardiovascular risk profile. In contrast, *SOD1* gene mutation carriers featured a higher cardiovascular risk profile, especially those carrying the more benign D91A and L118V mutations.

The higher cardiovascular risk profiles in *SOD1* gene patients are largely consistent with previous findings from the *SOD1* gene ALS mouse model. Fergani et al. found that presymptomatic ALS *SOD1* gene mice show lower cholesterol levels compared with wild-type mice, including HDL, very low density lipoprotein and LDL [26]. In addition, ALS mice at 65 days showed a delayed initial glucose clearance compared with wild-type mice, displaying

TABLE 4 Demographic and clinical characteristics of benign *SOD1* gene mutation carriers and other *SOD1* gene mutation carriers

	Benign *SOD1* gene mutation carriers ($n = 7$)	Other *SOD1* gene mutation carriers ($n = 14$)	*p*-value
Age (years)	68 (37–75)	42 (33–54)	0.03
Sex (male, %)	6 (86%)	8 (57%)	0.19
BMI (kg/m^2)	28.8 (27.5–30.0)	28.5 (27.2–29.8)	0.79
Fasting serum glucose (mg/dl)	111.5 (106.7–116.4)	102.1 (95.3–108.9)	0.05
SBP (mmHg)	126.2 (119.8–132.5)	129.8 (126.7–132.9)	0.37
TG (mmol/l)	1.5 (1.3–1.7)	1.4 (1.3–1.6)	0.62
TC (mmol/l)	4.2 (3.9–4.5)	5.6 (5.3–5.9)	**<0.001**
HDL (mmol/l)	1.0 (0.9–1.1)	1.3 (1.3–1.4)	**<0.001**
LDL (mmol/l)	2.7 (2.3–3.0)	3.8 (3.5–4.0)	**<0.001**

Note: Data are age-adjusted and represented as median (IQR) or *n* (%). Bold values indicate statistically significant results.

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; *SOD1* gene, superoxide dismutase 1; TC, total cholesterol; TG, triglycerides.

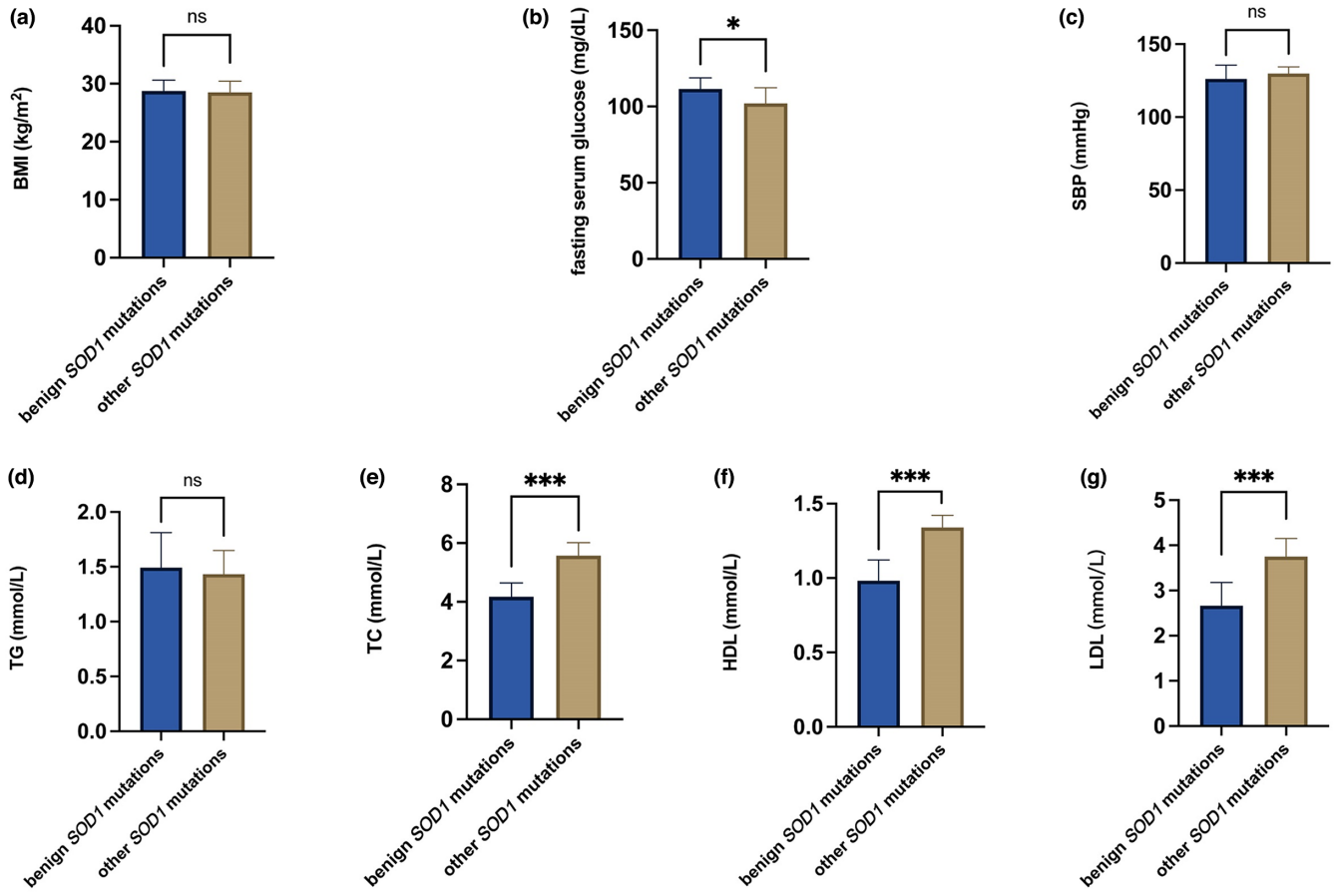

FIGURE 3 Metabolic factors in *SOD1* gene. Boxplots show mean and 95% confidence intervals for metabolic factors in presymptomatic carriers with benign *SOD1* gene mutations and presymptomatic carriers with other *SOD1* gene mutations. Asterisks mark significant (*$p < 0.05$) and highly significant (***$p < 0.001$) differences. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; *SOD1* gene, superoxide dismutase 1; TC, total cholesterol; TG, triglycerides.

higher glucose levels at 30 and 45 min [27]. These findings suggest that impaired glucose and lipid metabolism of *SOD1* gene mice can be detected even before the onset of symptoms. Furthermore, Dupuis et al. found that *SOD1* gene mice showed distinct metabolic alterations already during the presymptomatic stage, including reduced adipose tissue accumulation, increased energy expenditure and concomitant skeletal muscle hypermetabolism [28].

Previous studies evaluating the symptomatic phase of ALS suggested that high cardiovascular risk profiles were protective

prognostic factors in ALS. Whilst multiple studies reported a better prognosis in patients with higher BMI, associations between disease course and high lipids, high blood glucose levels and high blood pressure were only partially observed [24, 29–31]. Although the later course of the disease in presymptomatic carriers cannot be precisely predicted, it is well known that specific mutations are associated with a more aggressive or more benign disease course. *C9ORF72* gene-associated ALS is generally characterized by a later onset and faster disease progression [32]. Amongst *SOD1* gene patients, a greater heterogeneity has been reported, which is strongly linked to the specific mutation [33]. Specifically, patients with A4V mutations show fast disease progression [34], whilst the D91A and L118V mutations are associated with a comparatively benign disease course and longer survival [35]. Therefore, it may be hypothesized that a high cardiovascular risk profile in the presymptomatic stage is associated with a better prognosis in the clinical stage of ALS.

Consistent with this hypothesis, higher fasting serum glucose and lower HDL levels were found amongst *SOD1* gene carriers of benign mutations compared to other *SOD1* gene mutation carriers. However, not consistently, lower TC and lower LDL levels in benign *SOD1* gene mutation carriers were also found. Their relative benign clinical features could be related to compensatory mechanisms leading to such metabolic phenotypes. Normal *SOD1* gene protein is involved in lipid metabolism, mitochondrial respiratory repression and redox processes. Mutant SOD1 gene has an increased affinity to the anti-apoptotic protein Bcl-2 [36], which may contribute to an impaired capability to repress mitochondrial respiration and promote aerobic fermentation. An impaired mitochondrial Ca^{2+} buffering capacity and respiratory function was also related to mutant *SOD1* gene protein [37]. One study employing a human induced pluripotent stem cell model reported that impaired misfolded *SOD1* gene protein levels were higher in A4V compared to D91A mutant motoneurons [38] which may explain varying degrees of disruption of mitochondrial respiration, intracellular calcium and redox balance, and ultimately different metabolic profiles in ALS depending on the specific mutation. Considering the low number of patients in the *SOD1* gene subgroup analysis, these findings must be further explored in future studies.

So far, the underlying mechanisms linking metabolism with disease progression have not been fully understood. Hypermetabolism, as an important factor contributing to weight loss, has been described as a common phenomenon in ALS patients. It is hypothesized to be caused by alterations in the hypothalamus [39, 40] and/or by mitochondrial dysfunction [17, 41, 42]. The lateral part of the ventromedial nucleus of the hypothalamus affects food intake and energy expenditure. It is also involved in regulating insulin sensitivity, glucose and lipid metabolism. Atrophy of the hypothalamus [18] and (compensatory) increased levels of agouti-related protein mRNA have been demonstrated in ALS and are associated with glucose intolerance and enhanced appetite [18, 40]. Significantly enhanced energy intake and thereby blood glucose and lipid levels may provide energy support for repair mechanisms in the symptomatic as well as the presymptomatic stage of ALS.

Mitochondria, on the other hand, are essential for energy metabolism, and their dysfunction has been repeatedly described in early ALS pathogenesis [42, 43]. It has been shown that there was a linear correlation between the elimination rate of serum lactic acid and disease progression [44]. Studies based on animal models of ALS suggested that decreased ATP production in the central nervous system and muscles due to mitochondrial dysfunction was associated with decreased glycolysis and a switch towards lipids as the preferred source of energy in the muscle [27]. Increased storage of energy substrates in the presymptomatic stage of ALS may therefore indicate a compensatory mechanism to provide sufficient energy levels to counteract the pathological changes of the disease.

The following limitations have to be mentioned when interpreting the results of this study. First, our conclusions were drawn from cross-sectional analyses; therefore, presymptomatic gene carriers may display a heterogeneous burden of pathological changes, including some subjects who are close to the clinical onset of disease and others still far away. As our presymptomatic gene carrier cohort is followed up prospectively, future longitudinal data may provide additional insight. Secondly, despite using data from one of the largest presymptomatic gene carrier cohorts published to date, sample sizes for specific genes were still relatively small. Therefore, the results must be verified with a larger sample size.

CONCLUSION

In this study, distinct metabolic profiles in presymptomatic ALS gene carriers were identified, indicating a lower cardiovascular risk profile in *C9ORF72* gene and a higher cardiovascular risk profile in *SOD1* gene presymptomatic gene carriers compared to controls. Considering these findings in the context of the disease course to be expected for the present mutations, metabolic profiles might have a disease-modifying effect and prognostic value for presymptomatic ALS gene carriers. Additional studies and longitudinal follow-up are needed to further evaluate these findings.

AUTHOR CONTRIBUTIONS

Study conception and design: JD, ACL. Study supervision: DSF, JD and ACL. Acquisition and analysis of data: KX and JD. Writing of the manuscript: KX. Critical revision of the manuscript: SW, DF, JD and ACL. All authors contributed to manuscript revision, read and approved the submitted version.

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CONFLICT OF INTEREST

Nothing to report.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article.

ETHICS APPROVAL

This study was approved by the ethics committee of Ulm University, application number 68/19.

PATIENT CONSENT FOR PUBLICATION

Obtained.

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