Exploring the influence of smoking and alcohol consumption on clinical severity in patients with facioscapulohumeral muscular dystrophy

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Abstract

Despite the growing knowledge on the (epi)genetic background of facioscapulohumeral muscular dystrophy (FSHD), the substantial variability in disease severity that exists between FSHD patients is not fully understood. We hypothesized that smoking and alcohol consumption are disease modifiers in FSHD and contribute to the variability in disease severity, because they are both associated with higher levels of oxidative stress in muscle tissue. Oxidative stress is known to influence FSHD muscle tissue. One hundred and ninety-eight genetically confirmed FSHD patients completed a questionnaire from which the number of packyears of smoking and the lifetime cumulative alcohol units consumed were calculated. Disease severity was determined by the FSDH evaluation score. Multiple linear regression analyses showed that both the number of packyears and the amount of alcohol consumption did not influence disease severity (respectively B = 0.025, ΔR²=0.006, p = 0.231; and B = 0.000, ΔR²=0.004, p = 0.406). Although smoking and excessive alcohol consumption are unhealthy habits which should be discouraged, these results show that smoking and alcohol consumption have no clinically meaningful modifying effect on disease severity in FSHD patients. However, prospective data should show whether alcohol consumption and smoking influence disease progression rate.

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1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common inherited muscle disorders that causes weakness of the facial, shoulder girdle and upper arm muscles, often followed by axial and lower extremity muscle weakness [1]. FSHD is characterized by a highly variable disease course and severity, ranging from asymptomatic gene carriers to wheelchair bound patients.

FSHD is caused by wrongful expression of the DUX4 protein, which is toxic to muscle cells. The DUX4 gene is de-repressed in skeletal muscle cells of FSHD patients, due to relative chromatin relaxation of the D4Z4 macrosatellite repeat array on chromosome 4q35. This relative chromatin relaxation is caused by a repeat contraction of the D4Z4 array, with or without an additional mutation in a chromatin modifier gene, such as SMCHD1 [2,3]. Both D4Z4 repeat size and mutations in chromatin modifiers genes are known disease modifiers in FSHD [4]. However, these disease modifiers only partially explain the large variability in disease severity. Most likely, disease severity in FSHD is determined by a complex interplay of (epi)genetic, lifestyle and environmental factors [5].

Smoking and alcohol consumption may be two of these disease modifying lifestyle factors. Both have been associated with increased levels of oxidative stress in muscle tissue.
in the general population. In vitro studies have shown that in muscle cells of FSHD patients oxidative stress increases DUX4 expression and in turn, DUX4 expression increases the sensitivity of muscle tissue to oxidative stress [6-8]. In otherwise healthy smokers, increased levels of oxidative stress due to smoking seem to affect skeletal muscle tissue and consequently muscle function, showing reduced endurance of the quadriceps muscles [9-12]. Regarding alcohol, rat studies found that administering ethanol causes a significant increase in markers of oxidative stress in skeletal muscle [13]. Additionally, alcohol is known to adversely affect human skeletal muscle through a complex series of mechanisms, regularly leading to a metabolic myopathy in alcohol misusers [13,14].

As both smoking and alcohol consumption can increase oxidative stress levels in muscle tissue, and oxidative stress plays an important role in FSHD muscle pathology, we hypothesize that smoking and excessive alcohol consumption results in more severe muscle weakness. Therefore, in this study we assess the relationship between number of pack-years, alcohol consumption and disease severity in a large cohort of FSHD patients.

2. Methods

2.1. Patients

The data were collected in a large cross-sectional cohort study on FSHD (FSHD-FOCUS study) performed from 2014 to 2015 at the Neurology department of the Radboud University Medical Center, Nijmegen, the Netherlands [5]. The cohort consists of 203 genetically confirmed FSHD patients (type 1 and 2) aged 18 years and older and included both probands and familial cases.

2.2. Ethical approval

This study was conducted according to the principles of the Declaration of Helsinki (version October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The study received ethical approval from the regional medical ethics committee (NL48204.091.14). All patients provided written informed consent for study participation.

2.3. Disease severity

Disease severity was assessed using the FSHD evaluation score [15]. This score indicates clinical severity on a fifteen point scale, evaluating both strength and functionality of six separate muscle regions. Zero indicates no muscle weakness and fifteen indicates severe weakness in all body regions.

2.4. Smoking and alcohol use

Data on smoking and alcohol consumption were collected using a questionnaire [16]. Smoking and alcohol consumption status were categorized as never, former or current at the time of the study. Data regarding age at start and, if applicable, cessation of smoking and alcohol consumption were collected. The daily number of cigarettes smoked and weekly units of alcohol consumed were registered. Data regarding periods of increased or decreased smoking and alcohol consumption were collected and added to, respectively subtracted from, the total lifetime consumption. Lifetime smoking was expressed in pack-years (calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked; defining pack as 20 cigarettes). Additionally, data regarding smoking of other tobacco products was collected and converted into packyears: one cigar is taken as an equivalent of four cigarettes and one cigarillo or small cigar as equivalent of two cigarettes [17]. Lifetime alcohol consumption was expressed as the cumulative alcohol consumption. Finally, because of its potential anti-oxidant effect the cumulative red wine consumption was registered. Missing values were registered and deleted listwise.

2.5. Statistical analysis

Descriptive statistics were calculated for all outcomes and are presented as mean, SD and range unless stated otherwise. Multiple linear regression analysis was used to assess the influence of the number of pack-years and cumulative alcohol consumption on disease severity. The FSHD evaluation score, indicating disease severity, was used as the dependent variable. Independent variables were entered hierarchically: in the first block we added variables known to affect disease severity (age and D4Z4 repeat array size). In the second block we separately added the variables pack-years, cumulative alcohol- and red wine consumption. Subgroup analyses on heavy smokers and alcohol consumers were performed using analysis of covariance (ANCOVA), in which the FSHD evaluation score was used as the dependent variable and age and D4Z4 repeat array size were entered as covariates. Number of pack-years and cumulative alcohol consumption were entered as fixed factor. P-values are reported and statistical significance was defined as \( P < 0.05 \). \( R^2 \) values were used to describe the variance that was explained by the variables in both blocks. Statistical analyses were performed using SPSS version 25.

A sensitivity analysis was performed using G*Power statistical software version 3.1 [18]. We calculated the effect size this study should be able to detect in the context of multiple regression with a sample size of 196 patients that reported their lifetime smoking habits and 145 patients that reported lifetime alcohol consumption a power of 0.8 and \( \alpha \) of 0.05. The effect size is defined as Cohen’s \( f^2 \), in which \( f^2 \geq 0.02 \) represents a small, \( f^2 \geq 0.15 \) a medium and \( f^2 \geq 0.35 \) a large effect size. Cohen’s \( f^2 \) was calculated separately for smoking and alcohol consumption variables.
Table 1
Patient characteristics.

| Patient characteristics                  | n = 198 |  
|------------------------------------------|--------|
| Total cohort                             | 97 (49.0%) |
| Male sex (n)                             | 43 (21.9%) |
| Age (y) (mean, ±SD)                      | 51.1 ± 15.5 [range 18–84] |
| BMI (kg/m²) (mean, ±SD)                  | 25.4 ± 4.4 [range 15.4–40.8] |
| FSHD type (n)                            | FSHD type 1 = 187 (94.4%) |
| FSHD evaluation score (mean, ±SD)        | 6.8 ± 4.5 [range 0–15] |
| Smoking (n)                              | n = 196 (missing values = 2) |
| Smoking status (n)                       | 84 (43.0%) |
| Present smokers (n)                      | 43 (22.1%) |
| Former smokers (n)                       | 72 (36.7%) |
| Years stopped (mean, ±SD)                | 18.3 ± 14.2 [range 0–42] |
| Never (n)                                | 81 (41.3%) |
| Heavy smokers (>20 cigarettes used daily) (n) | 24 (12.2%) |
| Lifetime amount of packyears (mean, ±SD) | 9.8 ± 14.6 [range 0–99] |
| Smoking cigars (n)                       | 128 (65.5%) |
| Smoking cigarillos (n)                   | 13 (7.0%) |
| Cumulative alcohol consumption (mean, ±SD) | 8959 ± 14791.61 [range 0–97344] |
| Cumulative red wine consumption (mean, ±SD) | 2527 ± 5630 [range 0–35880] |

2.6. Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

3. Results

3.1. Patient characteristics

The total cohort study included 203 patients. Five patients were excluded because of partially missing data concerning exposure to smoking and alcohol. Patient characteristics and data on smoking and alcohol consumption are presented in Table 1.

3.2. Influence of smoking and alcohol consumption

Multiple regression analysis revealed that age and D4Z4 repeat array size (block 1) are significantly associated with clinical severity and explain approximately 23% of the variance in the FSHD evaluation score (p-values 0.000; Table 2). Adding the number of packyears in block two to the analysis did not yield any additional predictive value over block one (ΔR²=0.006, p=0.231).

Adding block two on cumulative alcohol consumption to the regression model instead of packyears showed no additional value to block one either (ΔR²=0.004, p=0.406) (Table 2), as did adding block two on cumulative exposure to red wine (ΔR²=0.013, p=0.131). Combining exposure to alcohol and smoking in block two of the regression model added a non-significant additional value of ΔR²=0.016 (p=0.263).

We assessed whether there was an effect only in present smokers, (n=43), which was not found (present smoking Block 1 R²=0.415, Block 2 ΔR²=0.04, p=0.131).

Next, we analyzed subgroups of heavy smokers and alcohol consumers to exclude the possibility of a subtle effect only detectable between subgroups at both ends of the spectrum (heavy users versus non-users). Using analysis of covariance we compared the group of heavy smokers, defined as the upper 20% of total in ‘average cigarettes used daily’ (39 patients; mean packyears 32.5±16.1 SD) to non-smokers (82 patients). Additionally, we compared the subgroup of excessive alcohol consumers, defined as the upper 20% of total in ‘average amount of units consumed weekly’ (29 patients; mean amount of units consumed weekly 16.3±9.0 SD) to zero-to-low amount alcohol consumers, defined as the lower 20% of total (29 patients). Both analyses showed no significant difference between these subgroups (smoking F(1114)= 0.928, p = 0.337; alcohol consumption F(1,52)=2.432, p = 0.125).

As FSHD1 patients with D4Z4 repeat array sizes of seven units or more show larger disease variability, we performed a subgroup analysis on patients with repeat sizes of seven units or more (n=85) and with six units or less (n=103). In both subgroups smoking and cumulative consumption of alcohol did not explain any additional variance (≥ 7 repeat units smoking block 1 R²=0.211, ΔR²=0.002; alcohol use block 1 R²=0.159, ΔR²=0.025; ≤ 6 repeat units smoking block 1 R²=0.267, ΔR²=0.012; alcohol use block 1 R²=0.291, ΔR²=0.001; red wine block 1 R²=0.274, ΔR²=0.014).

Additional subgroup analysis on sex did not show significant additional effects to block 1 (female sex smoking block 1 R²=0.262, ΔR²=0.027; alcohol use block 1 R²=0.289, ΔR²=0.000; red wine block 1 R²=0.291, ΔR²=0.000; male sex smoking block 1 R²=0.256, ΔR²=0.000; alcohol use R²=0.165, ΔR²=0.000; red wine R²=0.183, ΔR² =0.058).

3.3. Sensitivity analysis

The sensitivity analysis showed that using the 196 participants of our cohort with a known number of packyears, an effect size of f²=0.040 could be detected with a power of 0.8. Using the 145 participants with a known number of total alcoholic units consumed in our cohort, an effect size of f²=0.055 could be detected with the same power. This means that our cohort was large enough to detect a small effect (f²
between 0.02 and 0.15) of the lifetime exposure to smoking and alcohol consumption on disease severity.

4. Discussion

This study provides data on the lifetime exposure to smoking and alcohol consumption and their relation to disease severity in a large cohort of FSHD patients. We hypothesized that smoking and alcohol consumption influence disease severity in FSHD patients, based on the knowledge that both factors increase oxidative stress in muscle tissue and oxidative stress in turn leads to increased DUX4 expression. However, our data show no modifying effect of either smoking or alcohol consumption on clinical disease severity in FSHD.

This is the first study to examine the effect of smoking and alcohol consumption on the entire spectrum of disease severity in FSHD in a large cohort of FSHD patients. A study on disease penetrance evaluated the effect of smoking habits and alcohol intake in 61 FSHD gene carriers with D4Z4 repeat array sizes of six units or more, but found no difference between the affected and unaffected carriers, consistent with the results of our study [19].

The results of both studies are in contrast with our hypothesis. There are several possible explanations for the discrepancy between our hypothesis and our results.

First, it is possible that there is an effect of smoking and alcohol consumption on oxidative stress in FSHD patients, but that it is too small to influence disease severity or too small to measure in a rough clinical score, which makes the clinical relevance of such a small effect questionable. Second, the lack of effect found may be explained by an effect only existing in specific subgroups, in particular in groups with a higher lifetime exposure such as heavy smokers and/or alcohol consumers. We did not find an effect in these subgroups, although a small effect may go undetected as these group sizes were small.

Finally, it is possible that there is a difference in effects on muscles between drinking one alcoholic unit a day versus binge drinking or smoking one cigarette a day versus smoking heavily for a short period [20,21]. Because we used cross-sectional data, our results only show the cumulative lifetime effect of smoking and/or alcohol use on disease severity in FSHD and we cannot rule out a temporary effect of heavy smoking or binge drinking on disease severity. However, our results indicate that possible temporary effects of both heavy smoking and binge drinking do not influence long-term disease severity in FSHD patients.

Of note is that our study population showed a relatively high alcohol consumption compared to the general Dutch population (88.3% vs 74.5% respectively). The smoking rate of our cohort was above average as well: 21.9% versus 20.4% in the general adult Dutch population [22]. Prevalence of lifestyle risk factors increases in patients who experience health deterioration in general [23]. This has not been confirmed in neuromuscular patients, but, as FSHD patients often report health deterioration over time, this may explain the higher smoking and alcohol consumption rate in our cohort. In conclusion, this study did not find a clinically meaningful modifying effect of the lifetime smoking and alcohol consumption on disease severity in FSHD patients. Although smoking and excessive alcohol consumption are known to negatively affect health in numerous ways, they do not evidently pose a risk for more severe muscle weakness in FSHD patients [24,25]. However, prospective data are

### Table 2
Multiple regression analysis on smoking and alcohol consumption.

| Multiple regression analysis | R² | ΔR² | B    | β   | P-value |
|------------------------------|----|-----|------|-----|---------|
| **Block 1**                  |    |     |      |     |         |
| Constant                     | 0.231 | -   | 5.901 | 0.000 |         |
| Repeat size (units, n)       | -0.903 | -0.327 | 0.000 |     |         |
| Age (years)                  | 0.123 | 0.426 | 0.000 |     |         |
| **Smoking**                  |    |     |      |     |         |
| Block 1.2                    | 0.232 | -   | 5.727 | 0.000 |         |
| Constant                     | -0.877 | -0.320 | 0.000 |     |         |
| Age (years)                  | 0.123 | 0.430 | 0.000 |     |         |
| Repeat size (units, n)       | -0.863 | -0.315 | 0.000 |     |         |
| Smoking (packyears)          | 0.117 | 0.409 | 0.000 |     |         |
| **Alcohol consumption**      |    |     |      |     |         |
| Block 1.3                    | 0.206 | -   | 6.292 | 0.000 |         |
| Constant                     | -0.882 | -0.343 | 0.000 |     |         |
| Age (years)                  | 0.106 | 0.390 | 0.000 |     |         |
| Repeat size (units, n)       | 6.337 | 0.000 |       |     |         |
| Block 2.2                    | 0.210 | 0.004 | -0.916 | -0.356 | 0.000 |
| Constant                     | 0.113 | 0.416 | 0.000 |     |         |
| Repeat size (units, n)       | 0.000 | -0.069 | 0.406 |     |         |
| Cumulative alcohol consumption | |     |      |     |         |
needed to determine if smoking and alcohol consumption affect disease progression rates of FSHD patients.

**Declaration of Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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