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Lifetime endogenous estrogen exposure and disease severity in female patients with facioscapulohumeral muscular dystrophy

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Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is characterized by large variability in disease severity, that is only partly explained by (epi)genetic factors. Clinical observations and recent in vitro work suggest a protective effect of estrogens in FSHD. The aims of this study were to assess whether the lifetime endogenous estrogen exposure contributes to the variability in disease severity in female patients, and whether female patients experience changes in disease progression during periods of hormonal changes. We calculated the lifetime endogenous estrogen exposure by subtracting periods with high progesterone levels (in which estrogens are counteracted) from the reproductive life span. Multiple linear regression in 85 patients did not show a contribution of the lifetime endogenous estrogen exposure to disease severity (B = 0.063, P-value = 0.517, $\Delta R^2 = 0.003$). The majority of women reported an unchanged rate of disease progression through periods of hormonal changes, like menarche, pregnancy or menopause. Women that noticed differences reported accelerations as well as decelerations. These results indicate that differences in estrogen exposure do not have a clinically relevant modifying effect on disease severity. However, a clinically relevant protective effect of greater differences in estrogen levels, or a protective effect caused by a more complex interplay with other reproductive hormones, cannot be ruled out.

Keywords: Facioscapulohumeral muscular dystrophy; Reproductive hormones; Estrogens; Disease modifiers

1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is an inherited muscular dystrophy that initially affects the facial, shoulder and upper arm muscles, followed by the trunk and leg muscles [1]. It is caused by chromatin relaxation of the D4Z4 repeat array on chromosome 4q35, resulting in the misexpression of the *DUX4* transcription factor in myogenic cells, that finally leads to muscle cell death [2]. In its most common form, FSHD1, the chromatin relaxation is caused by a repeat contraction to 1–10 D4Z4 repeat units. In FSHD2, chromatin relaxation is in most cases the result of heterozygous mutations in the *SMCHD1* (Structural Maintenance of Chromosomes flexible Hinge Domain containing-1) gene in the absence of a repeat contraction [3]. One of the clinical hallmarks of FSHD is its large variability in disease severity, ranging from asymptomatic and minimally affected gene carriers to wheelchair-bound individuals [1]. Even within the same family, large differences in disease severity can occur, despite carrying an identical D4Z4 repeat array. Most variability is observed in families with longer sized repeat arrays (seven repeat units and more), in which asymptomatic gene carriers are common [4–6]. This unexplained variability strongly suggests that other, not yet identified, modifying factors must be involved in this disease. One of the longstanding questions in FSHD is whether sex-related modifiers could be identified that influence disease severity. Sex differences in disease severity in FSHD patients have been observed in different studies, consistently reporting a higher proportion of women among asymptomatic gene carriers [5–9]. Additionally, cases have been reported of women experiencing a persistent worsening of symptoms following pregnancies, early menopause or anti-estrogenic therapy [10–12]. However, limited work has been done to systematically assess changes in disease progression during periods of pronounced hormonal changes. Recently, in vitro studies showed that estrogens antagonize DUX4 activity and improve the differentiation properties of FSHD-derived myoblasts [10]. These findings

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suggest a protective effect of estrogens on disease severity in FSHD. We therefore hypothesized that a higher estrogen exposure in female patients could act as a disease modifying factor, resulting in less severe symptoms. In this study we assessed whether the lifetime endogenous estrogen exposure contributes to the variability in disease severity in female patients with FSHD. In addition, we evaluated whether female patients experienced subjective changes in the rate of disease progression during periods of hormonal changes.

2. Patients and methods

2.1. Patients

Data were collected in a large cohort study on FSHD (FSHD-FOCUS study) at the Neurology department of the Radboud University Medical Center, Nijmegen, The Netherlands from 2014 to 2015. The cohort consisted of 203 patients aged 18 years and older, including 105 female patients. All patients were genetically confirmed, including determination of the number of D4Z4 repeat units [2]. Women who underwent hysterectomy or ovariectomy were excluded, because of uncertainty of time of menopause and thus uncertainty of total estrogen exposure.

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 protocol was approved by the regional medical ethics committee. All patients signed informed consent.

2.3. Disease severity

Disease severity was determined using the FSHD clinical score. This is a fifteen-point sum score that evaluates severity of involvement of different muscle groups with zero indicating no symptoms and fifteen indicating severe involvement of all muscles groups [13].

2.4. Lifetime estrogen exposure

Data on reproductive history were collected using a questionnaire. The lifetime estrogen exposure was calculated as previously reported [14,15]. We calculated the reproductive life span by subtracting the age at menarche from age at menopause or from age at examination in pre-menopausal women. Next, we subtracted all periods with high progesterone levels from the reproductive life span, since progesterone counteracts the effects of estrogens. To calculate the unopposed estrogen exposure, the following periods with high progesterone levels were subtracted: pregnancies (including miscarriages), periods of oral contraceptive use and (post-menopausal) hormone replacement therapy, periods of breast feeding and the post-ovulation part of the menstrual cycle. The latter was calculated by taking fourteen days for every menstrual cycle (luteal phase), with the number of menstrual cycles being determined based on the woman's cycle length. Duration of pregnancy at a miscarriage was not recorded and we therefore subtracted three months for each of the 15 reported miscarriages as 80% of miscarriages occur in the first twelve weeks of pregnancy [14]. For ten out of 179 children, the duration of breast feeding child was unknown and we subtracted four months, the average time of breast feeding, for these children.

2.5. Subjective influence of hormonal changes

A second questionnaire addressed subjective changes in the rate of disease progression during periods of hormonal changes. Participants were asked to indicate whether their rate of disease progression accelerated, decelerated, or remained constant during menarche, menopause, and oral contraceptive use and during and after pregnancies.

2.6. Statistical analyses

Statistical analyses were performed using SPSS version 22. Descriptive statistics were calculated for both disease related and reproductive factors. Mean and standard deviation are reported unless stated otherwise.

Multiple linear regression analysis was used to assess the influence of the lifetime endogenous estrogen exposure on FSHD disease severity. The FSHD clinical score, a measure for disease severity, was the dependent variable. Independent variables were entered hierarchically. Block 1 contained variables known to influence disease severity, i.e. age and repeat length. In the second block, the calculated years of lifetime endogenous estrogen exposure was entered. B and P-values are reported, statistical significance is defined as $P < 0.05$. R^2 values were calculated to assess the additional variance that was explained by the lifetime endogenous estrogen exposure.

A sensitivity analysis was performed using G*Power statistical software [16] to calculate the effect size this study should be able to detect with the sample size of 85 women, with a power of 0.8 and α of 0.05. Effect size is given as Cohen's f^2, the proportion of variance uniquely accounted for by the lifetime endogenous estrogen exposure, in which $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium and large effect sizes respectively [17].

3. Results

3.1. Patient characteristics

Eighty-five women were included in this study. Of the 105 women participating in the cohort study, 103 returned the questionnaire. Thirteen women were excluded because they underwent hysterectomy and/or ovariectomy. Five women were excluded because age at menarche was missing. Baseline characteristics are presented in Table 1. None of the patients had received anti-estrogenic therapy.

3.2. Influence of lifetime estrogen exposure

Multiple linear regression revealed that age and repeat length are significantly associated with FSHD disease severity and explained approximately 29% of variance in disease severity (Table 2). Lifelong endogenous estrogen exposure was

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