ORIGINAL RESEARCH—PAIN

Polymorphisms of the Androgen Receptor Gene and Hormonal Contraceptive Induced Provoked Vestibulodynia

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ABSTRACT

Aim. Women who developed vestibulodynia (vulvar vestibulitis) while taking combined hormonal contraceptives (CHCs) and a control group of women were tested for polymorphisms of the gene coding for the androgen receptor (AR) that is located on the X chromosome.

Study Design. DNA from 30 women who developed vestibulodynia while taking CHCs and 17 control women were tested for the number of cytosine–adenine–guanine (CAG) trinucleotide repeats in the AR. In addition, serum-free testosterone was tested in both groups.

Results. The mean number of CAG repeats in the study group was significantly greater than the control group (22.05 ± 2.98 vs. 20.61 ± 2.19, respectively; $P = 0.025$). This significant difference persisted when analyzing the CAG repeats from the longer allele from each subject. Among those who were taking drospirenone-containing CHCs, the mean calculated free testosterone was 0.189 ± 0.115 ng/dL in the study group and 0.127 ± 0.054 ng/dL in the control group, all of whom were taking drospirenone-containing CHCs ($P = 0.042$).

Conclusion. In the study cohort, women who developed vestibulodynia while taking CHCs are more likely to have longer CAG repeats in the AR than women who took the same type of CHC but did not develop vestibulodynia. We speculate that the risk of developing CHC-induced vestibulodynia may be due to lowered free testosterone combined with an inefficient AR that predisposes women to vestibular pain. **Goldtein AT, Belkin ZR, Krapf JM, Song W, Khera M, Jutrzonka SL, Kim NN, Burrows LJ, and Goldstein I. Polymorphisms of the androgen receptor gene and hormonal contraceptive induced provoked vestibulodynia. J Sex Med 2014;11:2764–2771.**

Key Words. Dyspareunia; Vestibulitis; Provoked Vestibulodynia; Vulva; Vulvodynia

Introduction

Provoked vestibulodynia (formerly called vulvar vestibulitis syndrome) is the most common cause of painful sexual intercourse, affecting 12% of premenopausal women in the general population [1]. Provoked vestibulodynia is characterized by severe, burning/sharp pain that occurs in response to pressure applied to the vulvar vestibule. Dyspareunia (painful intercourse) is the defining symptom of provoked vestibulodynia. The results of research examining the underlying etiology of provoked vestibulodynia can be challenging to interpret and even contradictory. As the diagnosis of provoked vestibulodynia is based on signs and symptoms, not from a defined pathophysiology, it is likely that there are multiple causes of this disorder.

This study was conducted at the Center for Vulvovaginal Disorders, Washington, DC. Genetic analysis was performed at the Baylor College of Medicine, Houston, TX.

Recent studies have elucidated at least four possible distinct subtypes of provoked vestibulodynia: (i) provoked vestibulodynia secondary to hormonal changes [2–4]; (ii) provoked vestibulodynia secondary to neuroproliferation [5,6]; (iii) provoked vestibulodynia secondary to inflammation [7–9]; and (iv) provoked vestibulodynia secondary to hypertonic pelvic floor muscles [10,11].

One potential cause of hormonally mediated provoked vestibulodynia is use of combined hormonal contraceptives (CHCs). Several studies have shown that CHC use significantly increases the risk of developing provoked vestibulodynia by four- to 11-fold [2,12,13]. In addition, it has been demonstrated that CHCs induce morphologic changes in the vestibular mucosa, making it "more vulnerable to mechanical strain" [14]. Furthermore, CHC use decreases mechanical pain thresholds in women taking them [3].

Studies have noted decreased lubrication induced by CHCs [15]. During arousal, women become lubricated through a combination of vaginal transudate of serum from the submucosal vasculature and mucin secretion from the vestibular glands which include the Bartholin's, Skene's, and minor vestibular glands. These glands are the embryologic analogues of the Cowper's glands, prostate, and the glands of Littré in males. Consequently, in women, as in men, these mucin-secreting glands are androgen dependent [16]. It is well-known that CHC use leads to a reduction in serum-free testosterone (FT) by decreasing ovarian production of total testosterone and by inducing the liver to produce increased levels of sex hormone binding globulin (SHBG) [15]. In addition, some CHCs contain synthetic progestins that act as testosterone antagonists at the androgen receptor (AR) [17]. Therefore, we postulate that due to antiandrogenic effects, CHCs may cause dysfunction of the vestibular glands, which in turn could cause provoked vestibulodynia in some women.

Testosterone exerts its effects on gene expression through the AR. The AR gene is located on the X chromosome at Xq11-12. The amino terminal transactivating domain of the AR contains a highly polymorphic cytosine–adenine–guanine (CAG) trinucleotide repeat sequence and regulates androgen signaling in steroid hormone-sensitive cells [18]. This polymorphism in the AR gene ranges in size and may contain 11–32 repeats [19]. The length of the polymorphism is *inversely* associated with androgen-induced gene transcription [20]. Specifically, fewer CAG repeats are associated with high intrinsic AR activity (e.g., a more efficient receptor), and more repeats are associated with weak AR activity. In women, mutations and polymorphisms have been identified in the AR gene that are associated with various disorders including premature ovarian failure [21,22], ovarian cancer [23], and breast cancer [24]. Therefore, we further postulate that certain polymorphisms in the AR gene (i.e., AR with more CAG repeats) might predispose women to develop provoked vestibulodynia while on CHCs. This study was designed to differentiate between the number of CAG repeats in the AR in women who developed provoked vestibulodynia while taking CHCs vs. women taking CHCs who have not developed provoked vestibulodynia.

Study Design and Methods

This study comprised two groups of women: a study group and a control group. The study group consisted of 30 women with provoked vestibulodynia secondary to hormonal changes and who were referred by the faculty of the medical center. Women in this group developed provoked vestibulodynia while taking CHCs and had resolution of symptoms with cessation of CHCs and treatment with topical estradiol and testosterone applied to the vestibule (Figure 1). All women in this group were taking CHCs at their initial presentation. Of these 30 women, 21 were taking a CHC that contained the progestin drospirenone.

The control group consisted of 17 women who were currently taking CHCs containing the progestin drospirenone and who did neither complain of dyspareunia nor show any evidence of provoked vestibulodynia on physical exam. Subjects were recruited from healthy women coming for routine gynecological exams that included general medical history; reproductive history; breast examination; abdominal examination; inspection of the external genitalia, vagina, and cervix; bimanual examination of the uterus and adnexa; and appropriate testing which may have included a Pap smear, vaginal cultures, and testing for sexually transmitted infections. Control subjects were of similar age as the study group. No extraordinary measures were taken in recruiting this group as women using hormonal contraceptives commonly fall within this age range.

Women were excluded from the study if they had any known hormonal disease that might affect serum estradiol or testosterone, such as polycystic

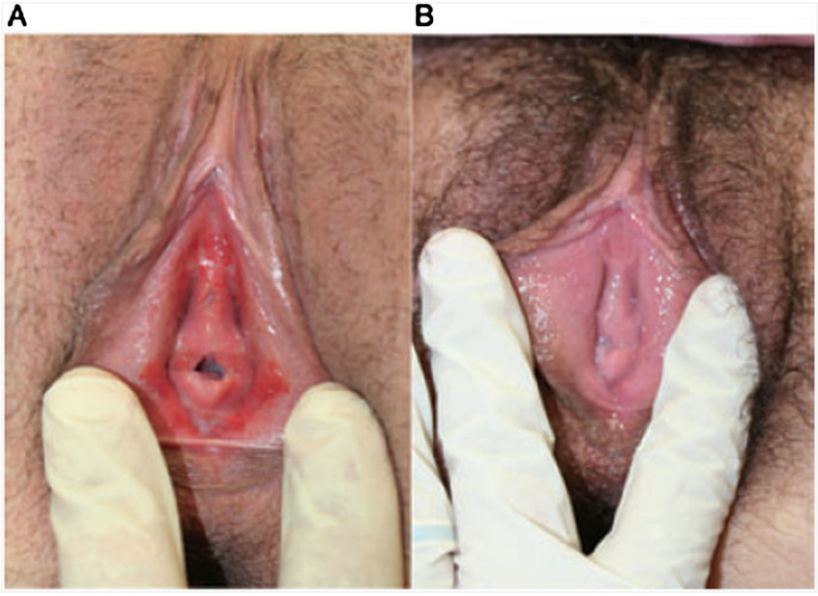

Figure 1 Vestibulodynia caused by a combined hormonal contraceptive: before estradiol and testosterone (A) and after estradiol and testosterone (B) treatments

ovarian syndrome or premature ovarian failure. Other exclusion criteria included diagnosis of vulvar dermatologic conditions including lichen sclerosus, lichen planus, psoriasis, lichen simplex chronicus, intraepithelial neoplasia or carcinoma, active vulvar or vaginal infection including candidiasis or bacterial vaginosis, prior vulvar surgery, hypertonic pelvic floor muscles, primary vestibulodynia, pregnancy, or lactation.

In both groups, blood was drawn for serum levels of testosterone, albumin, and SHBG (to calculate a FT) at initial presentation, and all samples were processed and assayed by Quest Laboratories. Total testosterone was determined by liquid chromatography tandem mass spectrometry, and free testosterone was determined by equilibrium dialysis. Calculated free and bioavailable testosterone values were based on the binding constants of testosterone to SHBG and albumin. SHBG concentration was determined by immunochemical luminescence assay, and albumin concentration was determined by spectrometric dye-binding assay. Study group subjects were taking CHCs and had provoked vestibulodynia upon enrollment; treatment was initiated after blood work for the study was obtained. Additional blood was drawn for DNA analysis. This study was approved by the Institutional Review Board at George Washington University Medical Center. Both control and study groups were informed about the overall objectives of the study, and consent for blood draws, genetic testing, and data collection was obtained from all patients participating in the study.

DNA was extracted from white blood cells with DNeasy Blood & Tissue Kit (69504, Qiagen, Valencia, CA, USA). The fragment containing the CAG repeat site in the AR gene was amplified by PCR (with primers: Forward 5-TCCAGAATCT GTTCCAGAGCGTGC-3, Reverse 5-GCTG TGAAGGTTGCTGTTCCTC-3). PCR conditions were as follows: annealing temperature 62°C, extension time 1 minute at 72°C, 40 cycles. PCR fragments of around 280 bp were purified by electrophoresis in 1.5% agarose gel and sent for sequencing (Lone Star Labs Genetic Sequencing, Houston, TX, USA). DNA sequencing data were read, and CAG repeat number on both alleles was recorded.

Data Analysis

The mean number of biallelic (both alleles) CAG repeats in each group was compared using the Mann–Whitney *U* test. Comparisons resulting in *P* values less than 0.05 were considered statistically significant. In addition, we compared the means of the longer alleles (CAG-L) in each group and included CAG-L data from a previously published database of 522 Caucasian women [25] as an external control group. The CAG-L data were compared by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test to assess differences between pairs of groups. testosterone and related measurements between the control population and women with provoked vestibulodynia were analyzed by unpaired *t*-test with Welch's correction for unequal variances. We

Table 1 Plasma testosterone and related values in study subjects

	Control group (n)	Study group (n)	*P* value
Albumin (g/dL)	4.5 ± 0.3 (13)	4.5 ± 0.3 (21)	0.7523
*SHBG (nmol/L)	316 ± 84 (13)	211 ± 115 (21)	0.0045
Total testosterone (ng/dL)	40 ± 11 (13)	37 ± 18 (21)	0.4896
*Bioavailable testosterone (ng/dL)	1.4 ± 0.6 (13)	2.3 ± 1.5 (21)	0.0339
*Free testosterone (pg/mL)	0.7 ± 0.3 (13)	1.0 ± 0.7 (20)	0.0438
*Calculated free testosterone (pg/mL)	0.127 ± 0.054 (13)	0.189 ± 0.115 (21)	0.0415
*CHC use (years)	6.2 ± 2.4 (12)	4.2 ± 1.2 (10)	0.0220
Age (years)	27 ± 4 (17)	29 ± 4 (21)	0.0810

The number of subjects included for each parameter is shown in parentheses as data were not available for all subjects. "CHC use" indicates patient-reported total duration of drospirenone-containing CHC use prior to onset of pain symptoms. Total testosterone was determined by LC/MS/MS, and free testosterone was determined by equilibrium dialysis. Calculated free and bioavailable testosterone values were based on the binding constants of testosterone to SHBG and albumin. SHBG concentration was determined by immunochemical luminescence assay, and albumin concentration was determined by spectrometric dye-binding assay. Comparisons between control and study groups were made by unpaired *t*-test with Welch's correction for unequal variances. Asterisks indicate statistical significance

CHC = combined hormonal contraceptives; LC/MS/MS = liquid chromatography tandem mass spectrometry; SHBG = sex hormone binding globulin

calculated the effects sizes (Cohen's *d*) of these differences to help understand the importance of these findings. A $d = 0.20$ is considered a small effect, $d = 0.50$ a moderate effect, and a $d = 0.80$ a large effect. All data are reported as mean ± standard deviation, and all analyses were carried out using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Twenty-nine of the 30 women in the study group and 16 of 17 in the control group were Caucasian. Mean age of each group was not significantly different (control group = 27 ± 4 years; study group = 29 ± 4 years; Table 1). Interestingly, the mean duration of CHC use was significantly shorter in the study group (Table 1). As shown in Figure 2, the frequency distribution of CAG repeat length for both alleles was shifted toward the shorter range in the control when compared with the study group. Indeed, the mean number of biallelic CAG repeats was significantly smaller ($P = 0.025$, $d = 0.53$) in the control group (20.61 ± 2.19) than in the study group (22.05 ± 2.98).

When only the CAG-L of each pair was examined, the frequency distribution curves exhibited a more distinct separation (Figure 3). As shown in the inset for Figure 3, the mean number of CAG-L repeats was significantly greater ($P < 0.05$) for the study group 23.73 ± 2.59 than either the internal control group (21.76 ± 2.11, $d = 0.81$) or an external control group from a previously published database [25] of 522 Caucasian women (22.4 ± 2.5, d = 0.53). The ANOVA *P* value for the CAG-L analysis was 0.011.

Lastly, we compared the mean calculated FT (cFT) of the control and study groups. However, we limited the data to those who were taking drospirenone-containing CHCs. This represented all of the control group and 21 women in the study group. Interestingly, the cFT for the control group was significantly lower ($P = 0.042$, $d = 0.69$) than the study group (0.189 ± 0.115 ng/dL vs. 0.127 ± 0.054 ng/dL, respectively). As shown in Table 1, bioavailable testosterone and free testosterone measured by equilibrium dialysis was also significantly lower in the control group compared with the study group. The main reason for this difference was apparently the higher SHBG levels in the control group (Table 1).

Discussion

The most important finding of this study is that women who developed provoked vestibulodynia while taking CHCs were more likely to have an AR gene with more CAG repeats than women who took the same type of CHC but did not develop

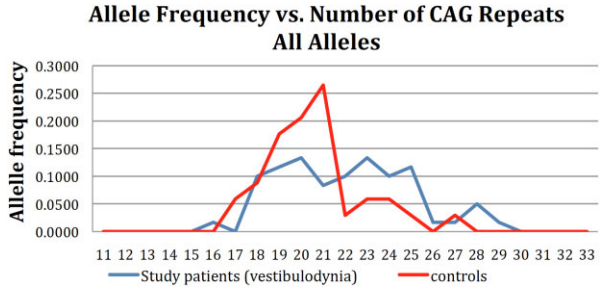

Figure 2 Cytosine–adenine–guanine (CAG) repeat allele frequency of all alleles

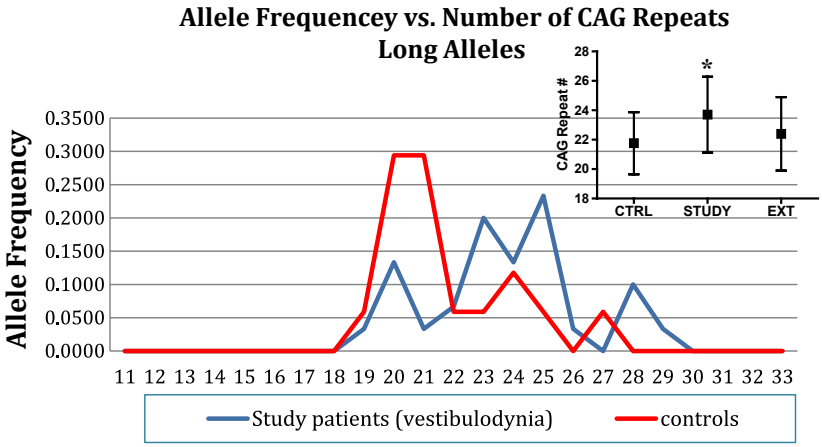

Figure 3 Cytosine–adenine–guanine (CAG) repeat allele frequency of long alleles. Inset: CAG repeat number (mean ± standard deviation [SD]) for the internal control group (CTRL), study group (STUDY), and external control group (EXT), as previously reported.[25] Data were compared by one-way ANOVA ($P = 0.011$), followed by Tukey's test for paired comparisons ($^*P < 0.05$).

provoked vestibulodynia. In addition, women who developed provoked vestibulodynia while taking CHCs may be more likely to have an AR gene with more CAG repeats than Caucasian women in the general population.

The effect size, as indicated by the calculated Cohen's *d* values, further suggests that the differences observed in our study are both significant and meaningful. In general, an effect size of 0.5 is considered moderate, and an effect size of 0.8 is considered large. The effect sizes in our study ranged from 0.53 to 0.81. In certain cases, while statistically significant differences between placebo and therapy could be questionable in terms of clinical meaningfulness, our study did not assess the efficacy of a therapeutic intervention. Instead, in two groups of women taking CHCs, the fact that one group was symptomatic (i.e., had vestibulodynia) and that this same group had significantly different CAG repeat lengths makes it self-evident that the difference in genotype is clinically meaningful (i.e., pain vs. no pain). Whether this is the most important factor remains unclear. Nevertheless, the effect size suggests that genotype exerts a measurable impact on the manifestation of vestibular pain symptoms in women taking CHCs.

Thus, our findings suggest that women who have more CAG repeats in their AR gene may be more likely to develop symptoms such as provoked vestibulodynia. This could be a consequence of the hormonal changes that women experience from taking CHCs. This finding is consistent with what is known about the AR and serum FT. Women with inefficient ARs (more CAG repeats) are more likely to develop symptoms from low FT than women who have more efficient ARs (fewer CAG repeats). Therefore, we hypothesize that the risk of developing CHC-induced provoked vestibulodynia is not solely due to the decrease in FT, but it is the *combination* of lowered FT with an inefficient AR that predisposes women to vestibular pain. This perspective could explain the reason that control patients had no vestibular pain in spite of having a significantly lower FT level than the study group. In fact, women in the control group had a significantly longer mean duration of CHC use by 2 years. Thus, CHC use alone is unlikely to predict vestibulodynia.

Consistent with our findings, other studies have shown a relationship between CHCs and provoked vestibulodynia. For example, in a prospective study, Bazin et al. showed that women who started taking CHC before the age of 17 were 11 times more likely to develop provoked vestibulodynia in comparison with women who had never taken CHCs [13]. In another prospective study by Battaglia et al., a group of healthy women who had no previous hormonal therapy for at least 6 months was administered drospirenone-containing oral contraceptives. Three months after beginning oral contraceptive use, women experienced increased pain during intercourse with decreased libido and spontaneous arousability, and diminished frequency of sexual intercourse and orgasm [26].

However, previous studies by Caruso et al. demonstrated a reduction in genital pain and overall improvement in sexual function associated with drespironone-containing oral contraceptives [27,28]. Yet these studies did not determine a specific cause for this improvement. Data were obtained from self-administered questionnaires and lacked any hormonal measurements or objective functional data, leaving open the possibility that the improvements in sexual function could have been due to amelioration of dysmenorrhea or symptoms of premenstrual syndrome. Further, a

large survey by Reed et al. did not find an association between CHC use and vulvodynia [29]. The most likely explanation for this disparity is that the constellation of symptoms that is used to define provoked vestibulodynia can be caused by several different pathophysiological mechanisms, all of which culminate in tenderness of the vulvar vestibule. The Reed study enrolled patients through phone interview, and data were collected by subsequent on-line or mailed questionnaires. There was no direct physical evaluation of patients and no qualification of severity of symptoms. In addition, the type of oral contraceptive used was not specified. Reed et al. acknowledged that additional research is needed and that there could be subgroups of women who may be affected by oral contraceptive use. Thus, the inconsistent findings between our study and those of Reed and Caruso et al. may be due to the different patient populations and methodologies used.

A strength of this study is that it assessed a specific subgroup of women with provoked vestibulodynia (i.e., those who only developed vestibulodynia after starting CHC) while excluding women with other possible causes of provoked vestibulodynia such as hypertonic pelvic floor muscles or women who always had provoked vestibulodynia. Therefore, it was more likely that the women in this study had provoked vestibulodynia induced only by CHCs and not by another cause. Analyzing the results of this study in combination with the findings of a recently published study that showed that women who developed provoked vestibulodynia while on CHCs were successfully treated with topical estradiol and testosterone [30] presents further evidence that provoked vestibulodynia may be induced by CHCs in a predisposed patient population.

In addition, it is common practice even among physicians to consider all forms of combined hormonal contraception as "*the pill*." However, CHCs are a vastly heterogeneous group of medications with different synthetic hormonal components in different dosages. Therefore, studies that do not control for the specific type of CHC can miss an association between specific types of CHCs and provoked vestibulodynia. This point is illustrated by Greenstein and colleagues who found that women taking CHCs containing only 20 μg of ethinyl estradiol were more likely to develop provoked vestibulodynia than women taking CHC with higher doses of ethinyl estradiol [31].

In this study, subjects who were taking CHCs with progestins other than drospirenone were excluded from the hormonal analyses because different progestins affect hepatic production of SHBG differently. Therefore, by only including subjects who were using drospirenone-containing CHCs, we were able to eliminate the potential for confounding effects due to the differences in the various progestin formulations found among the many available CHCs.

One weakness of this study is related to X-chromosome inactivation. Most women have random X inactivation, and therefore, the mean of their two alleles represents a very close approximation to their real gene expression. However, some women experience skewed X inactivation which has been shown to be important in many different diseases such as X-linked intellectual disability disorders [32]. In the past, X inactivation was studied by examining AR methylation as it was thought that if there was skewed X inactivation, the ratio would be consistent throughout the entire body. However, recent research has questioned this assumption, and it now appears that X inactivation can be tissue specific [33]. As the DNA in this study was obtained from lymphocytes, it was not possible to determine if skewed X inactivation might have an effect. To determine if skewed X inactivation does play a role, one would need to obtain vestibular biopsies in every study subject (an especially daunting prospect in women with provoked vestibulodynia) and determine the ratio of gene expression of the two alleles by RNA analysis. However, as the results of this study revealed an even greater separation between the two groups when comparing only the longer of the two alleles (Figure 3), it is certainly possible that skewed X inactivation of the shorter allele might further increase the risk of a woman developing CHC induce provoked vestibulodynia. Lastly, another potential weakness of this study is that almost all participants and controls were Caucasian. This was necessary because CAG repeat lengths vary significantly between different racial groups, but it may limit the applicability of our findings to other ethnicities.

In conclusion, our findings suggest that CHCs can induce provoked vestibulodynia in predisposed women. Our data suggest that one predisposing factor is an AR with an increased number of CAG repeats. Additional genetic studies should be done on this narrowly defined group of women to try to identify additional polymorphisms which may predispose them to develop provoked vestibulodynia secondary to CHCs.

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