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Distribution of CYP17α polymorphism and selected physiochemical factors of uterine leiomyoma in Barbados

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ABSTRACT

Uterine leiomyoma is a major reproductive health disease among women and in particular Black women. The present study sought to determine whether a single nucleotide polymorphism (SNP) of *CYP17* (rs743572) was associated with the risk of developing uterine leiomyoma (UL) in affected women in Barbados; a majority Black population. It also sought to determine if BMI, waist circumference and oestradiol levels were associated with UL in this group. A total of 96 random persons were assessed in a case–control study using a PCR-RFLP assay, and measurements of body mass index, waist circumference, and oestradiol levels were also assessed. Our results showed no genetic association with the risk of UL and this gene. The genetic distribution of *CYP 17α*- alleles resembled a normal Hardy–Weinberg distribution, and a relatively low risk of 0.25 at a confidence interval at 95%, of UL disease development. However, a significant association was found between oestradiol levels and fibroids, as well as oestradiol levels and BMI, at $P < 0.05$ among cases. Therefore our study indicates that significant associations between physiochemical factors comprising BMI, waist circumference, and oestrogen levels are disease indicators in this population. In conclusion, our findings suggest that obesity and its associated risk factors are important in a

Abbreviations: A, adenosine; ANOVA, analysis of variance; BFPA, Barbados Family Planning Clinic; BMI, body mass index; C, *cytosine*; *CYP17α*, cytochrome P450 17 alpha hydroxylase; dNTP, deoxyribonucleoside triphosphate; DNA, deoxyribonucleic acid; G, guanosine; PCR, polymerase chain reaction; RFLP, restriction-fragment length polymorphism; QEH, Queen Elizabeth Hospital; SNP, Single nucleotide polymorphism; T, thymidine; UTR, untranslated region(s); UL, uterine leiomyoma.

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majority Black Caribbean population, although the sample size needs to be increased.

Introduction

Uterine leiomyoma (UL) or fibroids are widespread benign tumours in women (Al-Hendy and Salama, 2006; Marshall et al., 1997; Othman and Al-Hendy, 2008; Wise et al., 2012). Enzymes in androgen biosynthesis have been targets for UL associations (Al-Hendy and Salama, 2006; Amant et al., 2004; Flake et al., 2003; Vieira et al., 2008). The Cytochrome P450-C17 alpha (*CYP17α*) gene codes for the enzyme 17 α-hydroxylase activity, a relatively early, but significant step in the biosynthesis of both androgenic hormones; oestrogen and progesterone (Sharp et al., 2004). The enzyme catalyses both hydroxylation at C17, followed by cleavage of the hydroxylated form between C17 and C20, to form dehydroepiandrosterone and androstenedione respectively (Sharp et al., 2004). *CYP17α* has a SNP (rs743572) in the 5′ UTR of the gene where a T, of the A 1 or wild type allele is converted to a C, the A2 or mutant allele (Othman and Al-Hendy, 2008; Sharp et al., 2004). *CYP17α* polymorphisms have also been investigated in populations with a sizeable ethnic diversity such as Brazil and South Africa (Amant et al., 2004; Rosa et al., 2008; Vieira et al., 2008), but not in the Caribbean region. However these studies were inconclusive in some populations. In South Africa, Amant et al. (2004) reported a higher risk for UL development in association with the mutant alleles (A2/A2) of the *CYP17α* genotype; but there was no such association with these alleles in Brazil (Vieira et al., 2008). It is against this background of conflicting data in Black populations, and the risk of UL associated with *CYP17α* alleles that the present study was undertaken. The present study therefore examines the distribution of CYP17α alleles and its genetic predisposition in UL in a predominantly Black Caribbean population with a high level of UL.

In the Caribbean, recorded incidence data on the condition is limited, although the condition is considered to be widespread. In Barbados, it is the fifth leading cause of hospitalisations in women of reproductive age for gynaecological conditions unrelated to pregnancy (pers. commun.). Epidemiological data on the condition in Barbados is lacking, however in Jamaica, it is approximately 33% (Fletcher et al., 2009), while in Trinidad and Tobago, a prevalence of 22% of fibroid cases with a high percentage of UL in Black women (91%) was reported (Uche-Nwachi et al., 2009). Barbados traditionally has less ethnic variety within its majority Black population than either Jamaica or Trinidad.

Several studies outside the Caribbean also report a greater predisposition to UL in Black populations, but indicate there is a lower risk of development in Caucasians (Al-Hendy and Salama, 2006; Denschlag et al., 2006; Ligon and Morton, 2000; Marshall et al., 1997). In the USA, data from a hysterectomy survey showed that 42% of hysterectomies were UL-related, with higher proportions of cases in women from southern states (Whiteman et al., 2008). Globally, a study of eight countries including the USA, Brazil, and Italy indicate a prevalence of approximately 10% (Zimmermann et al., 2012) while a recent study in Nigeria found a prevalence of 10% (Ezeama et al., 2012). Additionally, while several studies consistently show a higher incidence among Black women (Ligon and Morton, 2000), the global nature of the disease in different ethnic groups suggests several genes in various populations may be associated with UL (Eggert et al., 2012). Moreover, in Black women a racial admixture study by Wise et al. (2012) showed multiple genetic loci on three chromosomes which were responsible for UL. Although UL does not equally affect all ethnicities and is known to occur three times more in women of African ancestry (Marshall et al., 1997), UL prevalence and its genetics have not been established for any island in the Caribbean region, with a majority Black population.

The present study aimed to determine the frequency of the mutant allele and risk of UL in a Barbadian population by SNP genotyping in women affected with UL and women who were not affected with UL. We also assessed the relationship between body mass index (BMI), waist circumference, and oestrogen levels on UL presentation by correlation analysis. We also report an indication of the incidence of UL in Barbados based on a random survey of reproductive women in the population. This is the first study of its kind in Barbados related to SNP genotyping, and physiochemical indicators in UL development.

Materials and methods

Study population

Whole blood from 127 random samples of females from the Barbados Family Planning Association (BFPA), the QEH Gynaecology clinic and from a private Clinical Haematology laboratory in Barbados was collected from 2010 to 2012. These women were of reproductive age (20–50) and were grouped into two classes; UL-affected (cases) and non UL-affected (controls), as determined by their self-reporting on their disease status relative to fibroids, in a reproductive health clinic. Each participant visiting the clinic answered the question whether they had ever been diagnosed with fibroids. Questions and data on other gynaecological conditions or hormone based conditions were not included. Cases and controls were selected based on this self-reporting. There were 57 cases and 70 controls in total (see Table 1). The control group was selected based on the absence of fibroids only. All samples were collected with informed consent by the participants and protocols used were subject to an independent review and approved by the Ethics Board of the Queen Elizabeth Hospital (QEH) in Barbados.

Blood was collected from each study-participant, followed by two questions on age and race. Additional measurements of weight, height, and an oestrogen test for 73 participants were also made in 2011 to 2012. Oestrogen was measured by using an enzyme immunoassay (EIA) for quantitation of oestradiol (E2) test kit (Diagnostic Automaton Inc., USA) on corresponding serum from collected blood samples in 28 cases and 45 controls.

DNA extraction and genotyping

Genomic DNA was extracted from 200 μL of whole blood from each sample with the use of the QIAamp Blood DNA Midi Kit (Qiagen, Valencia, CA, USA). Each DNA sample was amplified using the following primers 5′-CAT TCG CAC TCT GGA GTC 3′-forward and 5′-AGG CTC TTG GGG TAC TTG-3′ reverse (Picado-Leonard and Miller, 1987). PCR amplification was conducted in a 25 μl reaction containing 200 ng DNA, as described by Vieira et al. (2008). Cycling conditions of the PCR reaction mixture, followed by overnight digestion at 37 °C in a 50 μl reaction with the restriction enzyme *Msp*A1 (Promega Inc., USA), and visualisation in 2% agarose gels under UV light was as described (Vieira et al., 2008). Genotypes were analysed on 96 samples in the study group.

Table 1
Clinical characteristics of samples in study group.

Variable	Cases (n)	Controls (n)	All samples (total)
Age			
<20	0	2	2
20–30	6	37	43
31–40	17	15	32
41–50	30	13	43
>51	4	3	7
Total	57	70	127
Race			
Black	56	67	123
Non-Black	1	3	4
Parity (no. of births)			
0	8	23	31
1	9	13	22
2	5	2	7
>2	4	2	6

Parity, oestradiol, BMI and waist circumference were measured for 73 samples in only one year.

Statistical analysis

All data were analysed using Sigma Plot version 11.0 (Systat Software Inc., San Jose, CA, USA). Statistical tests were carried out to determine statistical significance at $P \leq 0.05$, and Chi square analysis and Spearman's rank correlation coefficients were used where appropriate to test for associations. A multivariate ANOVA was used to compare multiple factors and Dunn's test was used to isolate differences between these means. Body mass index was calculated by using height and body mass. Waist circumference was measured in metres. Odds ratios (OR) were determined for the sample data at the 95% confidence level, between UL disease and BMI, and UL disease and allele frequencies. Additionally, allelic distribution was computed using the Hardy–Weinberg equation to determine the frequency distribution of each allele in the sample population.

Results

CYP17α genotypes

The general frequency of UL in the total sample population was 45% (Table 1) and the mean age of women in the study group was 35.5 ± 9.1 years (Table 2). Among the controls, the mean age was lower (32.0 ± 9.0 years) than the average age in the study group, while among the cases this was at least 6 years higher (41.5 ± 7.9 years) than the average age-group studied (Table 2). Among cases, the average number of children was 1.31 and among controls this was 0.86. However parity was low within the study group in general because each participant had on average 1 child.

The CYP 17 allelic distribution showed a preponderance of the homozygous dominant A1 alleles among both cases and controls in the study group (Fig. 1, Table 3). The A1 allele occurred approximately three times more frequently in the study population than the A2 or mutant allele (Table 3). Genotyping showed that the homozygous dominant genotype (A1A1) was also the most frequent in the study population, occurring at a frequency of 52% (Table 3). The frequency of the heterozygote genotype (A1A2) was 41%, while the mutant alleles (A2A2) were the least frequent, occurring at a frequency of only 6%.

Physiochemical UL indicators

A significant association between oestradiol levels and fibroids, as well as oestradiol levels and BMI, at $P < 0.05$ was obtained by multiple comparison of differences with Dunn's method (Fig. 2). Both oestradiol levels and BMI were found to be statistically significant at $P < 0.001$ in cases using one way ANOVA, but not among controls. Among the cases, the mean BMI was 25.3 ± 8.0 kg/m^2, while among controls there was a greater variation in BMI of 25.9 ± 11.7 kg/m^2. A larger variation in oestradiol levels was also seen in the cases when compared to the controls. The mean oestradiol levels in the control group was 344.4 ± 4.2 pmol/L when compared to cases which was 386.8 ± 38.8 pmol/L. Oestradiol levels of 200–400 pmol/L were associated with a BMI >25 kg/m^2 in cases (Fig. 2). Odds ratio for BMI with fibroid disease also showed low risk at 1.6, but a higher risk of 0.72 for oestradiol levels >200 pmol/L and UL (Table 3). Two way ANOVA also showed a significant association between oestradiol levels, BMI and fibroids at $P < 0.001$.

Table 2
Physiochemical clinical characteristics of study population.

Clinical characteristic	Means (±SD)[a]		
	Cases (N)[b]	Controls (N)	Average
Age	41 ± 7.9 (57)	32.1 ± 9.0 (70)	35.5 ± 9.8
Parity	1.4 ± 1.4 (26)	0.8 ± 1.16 (40)	1.0 ± 1.0
Body mass index (kg/m^2)	25.3 ± 8.0 (28)	25.9 ± 11.7 (45)	25.6 ± 10
Oestradiol levels (pmol/L)	386.8 ± 38.8 (28)	344.4 ± 4.2 (45)	348.6
Mean waist circumference (cm)	90.5 ± 12.0 (28)	82.8 ± 18.6 (45)	87.8 ± 12.9

[a] ±SD — standard deviation from the mean.
[b] N — number of samples.

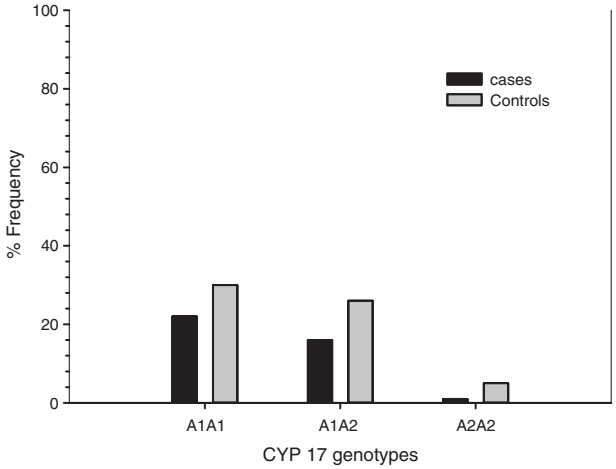

Fig. 1. Frequency distribution of CYP17 alleles in a random sample of Barbadian women.

Furthermore, among both cases and controls, there was a positive correlation between BMI and waist circumference of 0.5 using Spearman's rank correlation among cases and controls at $P < 0.05$.

Discussion

The main objective of this study was to determine the frequency of the *CYP 17α* allele in a Barbados female population of child bearing age, since it is believed mainly to be a disease related to hormone levels. In addition, the study also sought to determine the incidence of UL in Barbados through random sampling of a subset of the female population. Our study establishes the first record of UL frequency and some of its associated characteristics in Barbados. In general, the frequency of UL in Barbados from a random survey of women who were selected based on UL presentation at reproductive clinics, showed that approximately 42% of the participants presented with UL. This is higher than the global average reported for areas such as Brazil at 10% (Zimmermann et al., 2012). However, this compares favourably with African-American women in the Southern United States who presented with UL in the hysterectomy survey (Whiteman et

Table 3
Distribution of CYP17 genotypes, body mass index and oestradiol levels in the study group.

	Frequency		χ^2	OR (95% CI)[a]	*P*-value
	Cases	Controls			
	N (%)	N (%)			
Genotype			0.5	1.6	0.5
A1	29 (30)	41 (42)			
A2	8 (8)	18 (19)			
Alleles					
A1/A1	21 (22)	29 (30)	6.0		0.2
A1/A2	15 (16)	25 (26)			
A2/A2	1 (1)	5 (5)			
BMI					
BMI > 30 kg/m^2	5 (7)	6 (8)		1.4	0.5
BMI < 30 kg/m^2	23 (32)	39 (53)			
Oestradiol levels					
E2 > 200	13 (18)	26 (36)		0.7	0.5
E2 < 200	15 (20)	19 (26)			

[a] Odds ratio, 95% confidence interval.

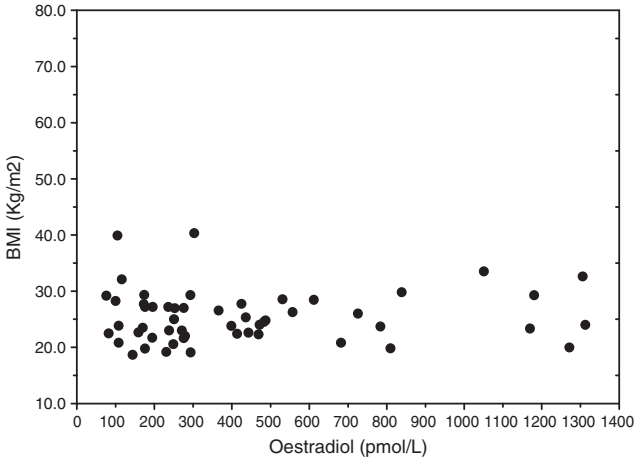

Fig. 2. Relationship between oestradiol levels and BMI among cases.

al., 2008). The Southern USA and Barbados share similar histories which may be reflected today by similar demographics and diet. Interestingly, the UL frequency reported here is almost twice that reported for Trinidad and Tobago, but similar to reports out of Jamaica of 33% (Fletcher et al., 2009). The population demographics although majority Black in Trinidad and Jamaica may be different from that of Barbados. It must be noted that these two studies represented only a subset of the female reproductive populations in both islands, targeting patients who also presented with other conditions such as polycystic ovarian syndrome and venous thromboembolism, in addition to uterine leiomyoma. Additionally, the frequency reported here would require further confirmation by an ultrasound report, although females in this study self-reported after seeing a gynaecologist.

The distribution of *CYP 17α* alleles in the Barbadian population indicated that the gene is in Hardy–Weinberg equilibrium. Since the population is in genetic equilibrium for the *CYP 17α* SNP (rs743572), the mutant allele is normally distributed and consequently the null hypothesis that the mutant A2 allele is significant in the Barbadian population sampled can be rejected. Similar results were reported for a sample Brazilian population for the risk associated with UL and *CYP 17α* genes (Vieira et al., 2008). Since UL is thought to be genetically heterogeneous within Black populations (Wise et al., 2012), especially in the diverse diaspora population, Black populations should not be treated as homozygous and may therefore present genetic variants that are not similar to each other in different countries.

UL gene expression is also conditioned by environmental factors (Flake et al., 2003; Han et al., 2008; Takeda et al., 2008). In this study, BMI and oestrogen levels were examined and these factors were shown to significantly impact Barbadian women affected with UL. Dietary factors have been reported to significantly affect more Black women with UL symptoms than Caucasians, while higher BMI and earlier age at menarche for Black women have also been previously reported in UL studies (Flake et al., 2003). Furthermore, while several reports have linked oestrogen levels to the presence of UL in women in some populations (Al-Hendy and Salama, 2006; Wise et al., 2012), oestrogen target genes, rather than oestrogen biosynthetic genes may be involved (Al-Hendy and Salama, 2006; Arslan et al., 2005). This may be consistent with the data on factors such as BMI and oestradiol levels also reported here and recent data suggesting that one of these targets, fatty acid synthase may have a key role to play in this disease (Eggert et al., 2012; Takeda et al., 2008). Although, higher proportions of Black women with fibroids have been reported (Baird et al., 2003; Hyuck et al., 2008; Marsh et al., 2007; Rosa et al., 2008; Taran et al., 2010), an ethnic factor for this disease is yet to be fully described. The current study however reflects previous reports of a normal distribution of *CYP 17α* among mainly Black women from a largely Black population (Rosa et al., 2008; Vieira et al., 2008). Although the *CYP 17α* mutant allele was not shown to be a significant predictor of the risk of UL disease development, other

cytochrome genes involved in oestrogen regulation and biosynthesis have been reported, but the sample sizes of Black women in those studies were small (Sharp et al., 2004).

This study represents the only known study on a predominantly Black population in the Caribbean in search of UL genes, and is a random observation of the female population in Barbados affected by UL. As such self-reporting of fibroids in this study was deemed to be reliable, since UL affected persons had previously seen a medical professional for UL in a gynaecological setting. Black women with UL are known to have larger tumours, so high BMI's could also be associated with a larger girth as a result of tumours rather than obesity; however the data is supported by previous assertions of links between UL, BMI and diet (Eggert et al., 2012; Flake et al., 2003). Therefore, studies incorporating larger proportions of Black women may give valuable insights into genes associated with fibroids in Black women in general, but genotype population studies should be considered in relation to environmental factors. These may impact gene expression since they may provide further insights into gene switches and regulation. In such a complex disease, these factors may play a role in genetic predisposition leading to expression by levels of oestrogen and obesity indicators.

Limitations to the study include the use of self-reporting for fibroids and the possibility of age being a confounding factor. Clinical presentation of fibroids is determined through an ultrasound by a physician. Patients in this study visiting both the QEH and the Barbados Family Planning Clinic had been seen by a physician previously for fibroids and may have had an ultrasound. In contrast, control patients were not seeing the physician for fibroids, but were in the clinic for other gynaecological investigations and may have had an ultrasound examination, but not in all instances. Since self-reporting in a clinical setting was used in this study, there may be some experimental bias of the results based on the self-reporting of patients. Additionally, since at least 20% of the controls and cases were in the reproductive age group, ages 31–40, most frequently associated with the diagnosis of UL (Baird et al., 2003; Flake et al., 2003), it was not seen as a limit to this present study, but is presented here as an epidemiological descriptor of UL in Barbados. The associations with waist circumference and obesity indicators in this study clearly need replication in a larger sample size with clinical confirmation by ultrasound. The small size of this study does not allow drawing of definite conclusions, but presents a preliminary view of fibroids in Barbados. In future work, a larger, matched, case–control study is necessary in order to answer whether this SNP is reproducibly associated with obesity traits in Barbados.

Conclusions

In conclusion our study established the incidence from a random sample of UL in Barbados and supports the conclusion that *CYP17* alpha, although related to oestrogen biosynthesis is not presently known as a risk gene for Black women with UL. Moreover it does support the view that obesity and oestrogen levels may be associated with UL, and that genes associated with obesity and oestrogen receptors should be further analysed in Black female populations.

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