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ORIGINAL ARTICLE

PROGINS Alu sequence insertion is associated with hyperprolactinaemia but not leiomyoma susceptibility

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Summary

Objective Leiomyoma and hyperprolactinaemia are both progesterone-dependent diseases. Hormone-related genes, such as the progesterone receptor (PGR), might be involved in their pathogenesis.

Design and measurements Subjects were divided into three groups: (i) leiomyoma ($n = 120$); (ii) hyperprolactinaemia ($n = 101$); (iii) normal controls ($n = 140$). We investigated the Alu (306-bp DNA) insertion in intron G of the PGR gene in all individuals. PGR gene polymorphisms [T1 (wild-type); T2 (PROGINS, with Alu insertion)] were determined by PCR and electrophoresis. Genotype and allele frequencies of the PROGINS in each group were detected and compared.

Results We observed no significant difference of the PGR*T1/T2 genotypes and allele frequencies between leiomyoma and other two groups. The proportions of T1 homozygote/heterozygote/T2 homozygote in each group were (i) 90/8·3/1·7%; (ii) 84·2/9·9/5·9%; (iii) 92·9/6·4/0·7%. In contrast, a higher percentage of T2-related genotype and allele were noted in hyperprolactinaemic women compared to other two groups. The proportions of T1/T2 alleles in each group were: (i) 94·2/5·8%; (ii) 89·1/10·9%; (iii) 96·1/3·9%.

Conclusions The PROGIN*T2-related genotype and allele are related to a higher susceptibility to hyperprolactinaemia. The PROGINS polymorphism is not associated with leiomyoma development.

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Introduction

Leiomyoma, the most common uterine neoplasm, occurs in around a quarter of women during their lifetimes.[1] The aetiology and related factors involved in the initiation and growth of leiomyoma remain obscure. Leiomyomas are thought to be monoclonal neoplasms.[2] The neoplastic transformation of myometrium to leiomyoma likely involves somatic mutations of normal myometrium and the complex interactions between multiple genes, hormone, growth factors, cytokines and environment. Leiomyoma is related to the auto- and paracrine interaction of sex-steroid hormone and local growth factors.[3–5]

There is increasing evidence indicating the involvement of progesterone in the pathogenesis of leiomyoma.[5] Both oestrogen and progesterone are recognized as promoters of leiomyoma growth.[6] Biochemical and immunocytochemical evidence has demonstrated the hormone receptors in leiomyoma cells.[7] Leiomyoma pathogenesis is a progestin-responsive process.[8] Volume decrease of leiomyoma in GnRH agonist-treated patients is associated with alternation in progesterone receptor (PGR) expression.[9]

Numerous hormones could influence the hypophyseal–gonadal axis, including oestrogen, progesterone and thyroid hormones (T3, T4, TSH).[10] Progesterone administration might suppress the hypothalamic–pituitary–ovarian axis, which further influences prolactin (PRL) production.[11] Hyperprolactinaemia might be induced and maintained by both oestrogen and progesterone administration.[10] Administration of progesterone might increase the level of PRL.[12] In contrast, progesterone also plays an inhibitory role in lactogenesis.[13] Progesterone could antagonize diethylstilbestrol-induced hyperprolactinaemia.[14]

The PGR has long been known to be essential for reproduction. The PGR content is related with the prognosis and survival for breast and ovarian cancers.[15,16] The PGR gene locates on chromosome 11q22–23.[17] A genetic polymorphism named as PROGINS has been identified in the PGR gene.[18] The satellite gene consists of a 306-bp DNA fragment insertion of the PV/HS-1 Alu subfamily in intron G.[18] It has been speculated that this insertion might results in the expression of an aberrant splice form of PGR, as it introduces a consensus splice acceptor site downstream of a consensus splice donor site.[18] Therefore, it is logical to suspect this polymorphism might be a candidate marker in the genetic study of disorders affecting female endocrine systems.

Genetic inheritability are related with numerous diseases, including leiomyoma and hyperprolactinaemia.[19,20] The identified SNP correlated with individual diseases might be plausible for understanding the related complex mechanisms. Westberg *et al.*[21] demonstrated that the PGR*G331A but not PROGINS is associated with

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PRL levels. Stevens *et al.*[22] also observed the correlation between PRL-1149 G allele and hyperprolactinaemia. Some polymorphisms might be related to leiomyoma development, including the androgen receptor[23] and oestrogen receptor.[24] In our previous surveys, we observed that oestrogen receptor and IGF2 gene polymorphisms likely contribute to the pathogenesis of leiomyoma.[25] In this study, we tried to evaluate whether PROGINS plays an aetiological or susceptibility role in leiomyoma and hyperprolactinaemia. It is among the first few surveys in these fields. Furthermore, to the best of our knowledge, it is the first report in Asians.

Patients and methods

Pre-menopausal Taiwanese women with leiomyoma, hyperprolactinaemia and normal controls were included. All women were divided into three groups: (i) leiomyoma ($n = 120$); (ii) hyperprolactinaemia ($n = 101$); (iii) normal controls ($n = 140$). This study was approved by the Ethical Committee of the China Medical University Hospital. Informed consents were signed by all women who donated their blood. There were no significant differences between each group in age, weight and height.

Leiomyoma was diagnosed by ultrasonography and confirmed by pathological examination after myomectomy or hysterectomy. Hyperprolactinaemia was diagnosed on the basis of chronic irregular menstruation with elevated levels of serum PRL (> 600 mU/l) without secondary causes of hyperprolactinaemia, including primary hypothyroidism (elevated TSH with low free T4) and drug use (e.g. dopamine receptor blockers such as phenothiazines or the antiemetic metoclopramide). All individuals with hyperprolactinaemia were on medical therapy with the bromocriptine (parlodel).

Total cellular DNA was extracted from peripheral blood and subjected to analysis by PCR and gel electrophoresis of the PCR products. According to GenBank (Accession No. Z49816) and the previous report of Rowe *et al.*,[18] primers were designed as follows: forward primer, 5′GGCAGAAAGCAAAATAAAAAGA3′; reverse primer, 5′AAAGTATTTTCTTGCTAAATGTC3′. The PCR reaction was carried out in a total volume of 25 μl containing 100 ng of genomic DNA, 2 pmole of each primer, 1 × Taq polymerase buffer (with 1·5 mM $MgCl_2$), and 0·5 unit of Taq DNA polymerase (Promega, Madison, WI, USA). PCR amplification was performed in a programmable thermal cycler PTC-100™ (MJ Research, Waltham, MA, USA). Cycling condition was set as follows: on cycle at 94 °C for 5 min, 35 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 45 s and one final cycle of extension at 72 °C for 3 min.

The PCR products were applied to electrophoretic analysis with the use of a 2% agarose gel (Fig. 1). Two DNA fragments could be detected: a 159 bp DNA fragment representing 'T1' allele (wild-type) and a 465 bp DNA fragment representing 'T2' allele (PROGINS, with a 306-bp of PV/HS-1 Alu insertion). The DNA fragments were also cloned for sequence verification by DNA sequencing. Each allele of PGR gene was recognized according to its size. Genotypes and allelic frequencies for T1/T2 in each group were compared. Allelic frequencies are expressed as a percentage of the total number of alleles. Differences in the frequencies of the alleles between each groups were analysed by χ^2 test. A P-value < 0·05 was considered statistically significant.

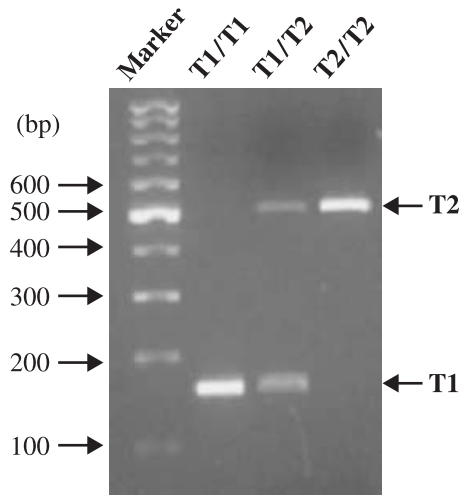

Fig. 1 Photography of an agarose gel stained with ethidium bromide to resolve the 306 bp intron G insertion polymorphism of the progesterone receptor gene. The 159-bp band represents the wild-type allele (T1 allele) and the 465-bp band represents the mutant allele (T2 allele). Lanes marked as T1/T1, T1/T2 and T2/T2 show the homozygous wild-type, heterozygous and homozygous mutant patterns, respectively.

Table 1. Genotypes and allele frequencies of PROGINS gene polymorphisms in women with hyperprolactinaemia, leiomyoma and normal controls

Genotypes	Leiomyoma $n = 120$ (%)*'‡	Hyperprolactinaemia $n = 101$ (%)†'‡	Controls $n = 140$ (%)*'†
T1/T1	108 (90)	85 (84·2)	130 (92·9)
T1/T2	10 (8·3)	10 (9·9)	9 (6·4)
T2/T2	2 (1·7)	6 (5·9)	1 (0·7)
Alleles	$n = 240$ (%)§'**	$n = 202$ (%)¶'**	$n = 280$ (%)§'¶
T1	226 (94·2)	180 (89·1)	269 (96·1)
T2	14 (5·8)	22 (10·9)	11 (3·9)

*'‡Nonsignificant difference; †p-value quals; 0·032; §'**Nonsignificant difference; ¶p-value = 0·003.

Results

The proportions of PGR genotype and allele frequencies in leiomyoma, hyperprolactinaemia, and normal controls group are listed in Table 1. Most individuals appeared to be T1 homozygotes. We observed significant difference of the PGR*T1/T2 genotypes between leiomyoma and other two groups. In contrast, a higher percentage of T2-related genotypes was noted in hyperprolactinaemic than patients in normal controls (P-value = 0·032, Table 1). Allele frequencies for T1/T2 between the leiomyoma and other two groups were nonsignificantly different. In contrast, compared to normal controls, a higher proportion of T2 allele was noted in the hyperprolactinaemic individuals (P-value = 0·003, Table 1).

Discussion

Traditionally, oestrogen has been considered to be the major stimulus to leiomyoma growth. However, some biochemical, histological

Table 2. Correlations of progesterone receptor (PGR) gene polymorphism with individual diseases

Correlation		Non-correlation	
PGR SNP locations	Diseases	PGR SNP locations	Diseases
PROGINS	Endometriosis,[41,44] ovarian cancer with diethylstilbestrol exposure,[50] breast cancer,[42] obesity[51]	PROGINS	Leiomyoma,[8] PRL level,[21] ovarian cancer,[52,53] panic disorder,[54] breast cancer,[39,55] idiopathic recurrent miscarriage[56]
G331A	Prolactin level,[21] implantation failure,[46] breast cancer,[45] endometrial cancer,[57] panic disorder[54]	G331A	Breast cancer[58]
G1031C (Ser344Thr), G1978T (Leu660Val), C2310T (His770His)	Recurrent abortion[59]	G1978T (Leu660Val)	Breast cancer,[60] ovarian cancer[61]
G331A, C2310T (His770His)	Implantation failure[46]		
G1978T (Leu660Val)	Breast cancer[60]		

and clinical evidence has highlighted the equal contributions of progesterone and oestrogen upon leiomyoma tumour genesis.[26] Some investigations have suggested that progesterone and PGR play important roles in the modulation of mitotic activity, local growth factors and growth factor receptors, as well as other paracrine mechanisms in leiomyoma development.[27] Furthermore, the PGR can suppress oestrogen receptor signalling as well as the stimulation of leiomyoma growth.[28] Compared to the PGR in myometrium, the PGR in the leiomyoma is more resistant to suppression by gonadotrophin releasing hormone analogus.[29]

Progesterone is known to influence the release of PRL.[21] Administration of progesterone can raise PRL secretion.[10,12] The stimulatory influence of progesterone on pituitary PRL release is mediated by PGRs within the brain.[21] Intermediate factors include β-endorphin,[30] serotonin,[31] or dopamine.[32] Through PGRs in the brain, progesterone might react with these intermediate factors, which further stimulate PRL production.[12] Progesterone could up-regulate PRL gene and subsequent PRL expression.[33] The PGR could regulate PRL expression during the differentiation processes of endometrial stromal cells.[34] Furthermore, cooperative transcriptions and regulations between PRL and PGR have been observed.[35] During the neonatal phase, the development of the uterus and endometrium involves the combined coordination of progesterone, oestrogen and PRL receptors.[36]

Numerous disorders, such as leiomyoma, hyperprolactinaemia and endometriosis, have been attributed to genetic susceptibility. Genetic studies of multifactorial diseases such as leiomyoma and hyperprolactinaemia are difficult to approach due to the uncertainty of a polygenic trait. Despite polymorphisms not being directly linked to a certain disease, they are useful tools in the study of multifactorial disorders.[37] The gene encoding the PGR might be a useful candidate for screening leiomyoma.[8] The PGR gene is located in chromosome region 11q22–23, which is a proposed site of tumour suppressor genes and a site of loss of heterozygosity (LOH) in women with endometriosis[38] and breast cancer.[39] This mutation results in an amino acid substitution in the hinge region of the PGR and alters the function of the receptor.[40]

Numerous diseases are related with PGR gene polymorphisms (Tables 2 and 3). The 306-base pair insertion polymorphism in intron G of the PGR is related to the development of endometriosis.[41] Wang-Gohrke *et al.*[42] suggested a dosage effect of PROGINS upon the decreased risk for breast cancer. High levels of PGRs might be associated with better survival.[43] Recently, Lattuada *et al.*[44] demonstrated that the PROGIN *T2 allele is associated with a twofold risk of developing endometriosis. Westberg *et al.*[21] also demonstrated the correlation between PGR 331*A-related genotype and higher production of PRL. Increased production of PGR-B by the G331A polymorphism might predispose women to breast cancer development through increased PGR-B-dependent stimulation of mammary cell growth.[45] Possession of the PGR 331*A and H770H*C alleles for PGR polymorphism are associated with an increased risk for implantation failure postembryo transfer.[46]

In this study, we observed that the genotype and allele frequencies for PGR T1/T2 between the leiomyoma and control groups were not significantly different. In contrast, we observed a statistically significant association between the PROGINS genotype and hyperprolactinaemia. The PGR*T2-related genotype and allele are related to a higher susceptibility to hyperprolactinaemia. We also observed that the PROGIN proportions in Asians were not compatible with those of Caucasians reports (Table 3). A lower percentage of T2-related genotypes are seen in Taiwanese population. These discrepancies might be due to numerous factors, including racial variation, illness classification, environmental variation, and multiple enzymatic processes and interactions.

The mechanisms of SNPs upon individual diseases remain uncertain. Despite the fact that SNPs do not alter the transcript products, some investigators have demonstrated that the disequilibrium effects of certain genotypes might influence the related efficiency of the transcripts.[47–49] Presumably, the distinct biological condition caused by PROGINS is among the numerous contributions which influence the development of hyperprolactinaemia. These contributions include genetic, dietary, and environmental regulating hormonal and nonhormonal conditions. Furthermore, the gene dosage effect of PROGINS with other hormone gene polymorphisms upon individual hormone-related disease might be considered.[42] The PGR polymorphisms might be in linkage disequilibrium with other unidentified functional polymorphisms, which cooperatively influences the susceptibility to hyperprolactinaemia.

Table 3. Genotypes and allele frequencies of PROGINS gene polymorphisms in previous reports

Authors		T1 homozygote (%)	T1/T2 heterozygote (%)	T2 homozygote (%)	*P*-value
Massart *et al.*, 2003[8]	Leiomyoma	70·7	24	5·3	NS
	Normal controls	71·1	26·2	2·7	
Westberg *et al.*, 2004[21]	Hyperprolactinaemia	74·9	22	3·1	*
Lattuada *et al.*, 2004[44]	Endometriosis	67·9	29·8	2·3	0·041
	Normal controls	78·7	20·5	0·8	
Wieser *et al.*, 2002[41]	Endometriosis	68·4	28·4	3·2	0·004
	Normal controls	85	14	0.9	
Tong *et al.*, 2001[53]	Ovarian cancer	73·9	22·1	4	NS
	Normal controls	72·7	26·8	0·5	
Kurz *et al.*, 2001[56]	Idiopathic recurrent miscarriage	73·6	23·2	3·2	NS
	Normal controls	79.7	19	1·3	
Donaldson *et al.*, 2002[55]	Breast cancer	73·9	21·7	4·4	NS
	Normal controls	68·3	26·7	5	

*No controls group; NS, nonsignificant difference.

In conclusion, our data suggest that PROGINS is associated with the susceptibility of hyperprolactinaemia. In contrast, PROGINS is not correlated with increased susceptibility to leiomyoma. However, the use of PROGINS as a marker for predicting susceptibility to hyperprolactinaemia remains further investigation. Larger series are required to confirm these observations and to further examine the interaction between PROGINS and hyperprolactinaemia development. Furthermore, other hormone gene polymorphisms in leiomyoma and hyperprolactinaemia also need to be investigated.

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