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Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17

Adam H.Carey[1,3,*], Dawn Waterworth[1], Kirty Patel[2], Davinia White[3], Julie Little[1], Patricia Novelli[1], Stephen Franks[3] and Robert Williamson[1]

[1]Department of Molecular Genetics, [2]Unit of Metabolic Medicine and [3]Department of Obstetrics and Gynaecology, St Mary's Hospital Medical School, Imperial College of Science Technology and Medicine, London W2 1PG, UK

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Fourteen Caucasian families with 81 affected individuals have been assessed in which polycystic ovaries/male pattern baldness (PCO/MPB) segregates as an autosomal dominant phenotype (1). The gene CYP17, coding for P450c17α (17α-hydroxylase;17/20 lyase) on chromosome 10q24.3 is the rate-limiting step in androgen biosynthesis. We have identified a new single base change in the 5′ promoter region of CYP17 by heteroduplex analysis. This creates an additional SP1-type (CCACC box) promoter site, which may cause increased expression. This base change also creates a recognition site for the restriction enzyme *Msp*A1 allowing a simple screening procedure. There is a significant association between the presence of this base change (A2) and the affected state for consecutively identified Caucasian women with PCO as compared either to consecutively matched controls (*P* = 0.03) with an odds ratio for those with at least one A2 allele of 3.57, or to a random population (*P* = 0.02) with an odds ratio of 2.50. Within the fourteen families, members with PCO or MPB have a significant association with the occurrence of at least one A2 allele compared to their normal relatives, with an odds ratio of 2.20 (*P* = 0.05). The base change does not co-segregate with the affected phenotype within the families showing association, demonstrating that this mutation of CYP17 does not cause PCO/MPB. Variation in the A2 allele of the CYP17 gene is a significant factor modifying the expression of PCO/MPB in families where it has been demonstrated to segregate as a single gene disorder, but it is excluded as the primary genetic defect.

INTRODUCTION

Polycystic ovary syndrome is a highly prevalent endocrine disorder which is the most common cause of anovulatory infertility and hirsutism (2–4). Polycystic ovaries and premature male pattern baldness have been demonstrated to be the female and male phenotypes of a condition that segregates, within families of different racial backgrounds, with an autosomal dominant mode of inheritance and close to full penetrance (1). This suggests that the underlying abnormality within each family is caused for by a single gene defect, although more than one gene may be responsible for PCO/MPB in the population. The involvement of different genes in different individuals is an attractive hypothesis, as it might explain the variable clinical phenotypes that occur in association with the ovarian morphology. The variable presenting symptoms include menstrual disturbances, hirsutism, acne, and infertility, occurring alone or in combination.

Despite the variable clinical presentation, there is a consistent biochemical finding of an elevation of serum androgens (5,6). Men with premature male pattern baldness, identified from families of women known to have polycystic ovaries, also have shown an elevation of their serum androgens when compared to age and weight matched controls from the same pedigrees (7). This supports the hypothesis that polycystic ovaries and premature male pattern baldness are caused by a common underlying disorder of androgen biosynthesis or metabolism.

Previous studies have suggested that there is abnormal regulation of the enzyme P450c17α in women presenting with polycystic ovaries (8,9). This enzyme catalyses the rate limiting step in androgen biosynthesis in both the ovary and adrenal gland, and therefore the gene coding for CYP17 is a candidate gene for PCO/MPB. We have analysed the segregation of CYP17 with PCO/MPB in fourteen Caucasian pedigrees.

RESULTS

A PCR fragment of 459 bp was generated using primers designed from the published sequence (10) of the 5′ region of CYP17. Heteroduplex analysis of the PCR product identified the presence of a single base change which creates an SP1-type (CCACC box) promoter site in which a T is replaced by a C at −34 bp from the initiation of translation from the published sequence (Fig. 1).

This also creates a recognition site for the enzyme *Msp*A1. We have designated the published sequence as the A1 allele and the mutated allele as A2. When the PCR product is screened using the restriction enzyme *Msp*A1, the presence of the base change in both alleles (homozygous A2 individuals) will generate fragments of 124 and 335 bp. Heterozygous individuals will have three fragments present, of 459, 335 and 124 bp. A homozygous (A1) individual would only demonstrate the uncut PCR product of 459 bp.

*To whom correspondence should be addressed

		Met
-60	-34	0

GAGTTGCCACAGCTCTTCTACTCCAC/T/GCTGTCTATCTTGCCTGCCGGCACCCAGCCAGCCACCATG

Mutation /C/

Figure 1. The sequence of the 5′ region of CYP17 showing the proposed initiation site of translation, the −34 bp mutation site where a T is replaced with a C and the proposed initiation site of translation at −60 bp (10).

Of 71 probands recruited into this study, 44 were Caucasian, and full screening for the base pair change was possible for all first degree family members in 20 pedigrees (n = 196), of whom 14 pedigrees were Caucasian (n = 142). In these pedigrees 13 family members were excluded from the association analysis as the affection status could not be determined according to our criteria. Thirty three women (of whom 24 were Caucasian) known to have normal ovarian morphology and 117 individuals of unknown affection status have also been screened. The results of screening are summarised in Table 1.

The prevalence of A2 in the Caucasian probands is significantly greater than in either the normal Caucasian control group of women known to have regular cycles and normal ovaries (P = 0.03, with an odds ratio for the presence of at least one A2 allele of 3.57), or the ethnically matched reference population (P = 0.02, with an odds ratio of 2.50). There was no difference between the two control groups in the prevalence of the A2 allele. In the 14 Caucasian families the prevalence did not differ from that of the consecutively identified probands. However, the prevalence differs between affected and unaffected family members (P = 0.05, with an odds ratio of 2.2). There is no difference in these analyses when all probands of different ethnic backgrounds were included. The incidence of the A2 allele in probands, and women with normal ovaries, of all races is very similar to that seen in the Caucasian population.

Pairwise lod scores between the disease locus and the A2 allele demonstrated neither linkage nor exclusion in any families (Table 2).

The chromosomal order and interval distances of D10S185, D10S198, D10S192, D10S221 and D10S209 microsatellite markers have been confirmed by marker−marker pairwise analysis and are shown in Figure 2. Pairwise lod scores between the disease locus in the fourteen Caucasian pedigrees (and all screened pedigrees) and the microsatellite markers are summarised in Table 3. Taking a lod score of < -2 as evidence of exclusion, the disease locus can be excluded from a 12 cM interval flanking D10S185 and a 23 cM interval flanking D10S221.

A location map summarising lod scores for PCO/MPB in these pedigrees at recombination fractions either flanking or within the intervals D10S185, D10S209 is illustrated in Figure 3. The maximum negative lod score generated between D10S192 and D10S221 for the Caucasian families was −72.69 and for all families was −88.68. These data exclude the presence of a mutated gene which causes PCO/MPB from this region of the long arm of chromosome 10 within these pedigrees.

The lod scores generated from either the Caucasian pedigrees or all pedigrees for either the two point or multipoint analyses only differ in the degree of the result. This would be consistent with our previous observations that the mode of inheritance of PCO/MPB is not significantly different in families of a variety of ethnic backgrounds.

Table 1. Results of screening for the presence of the A2 allele

	A2 Present	A1 Present	% A2 Present
All probands	49	22	69%
Caucasian probands	30	14	74%
All women with normal ovaries	15	18	45%
Caucasian women with normal ovaries	9	15	38%
Reference population	54	63	46%
Caucasian affected family members	56	25	69%
Caucasian unaffected family members	24	24	50%
Caucasian MPB male family members	17	8	68%
Caucasian normal male family members	6	7	46%

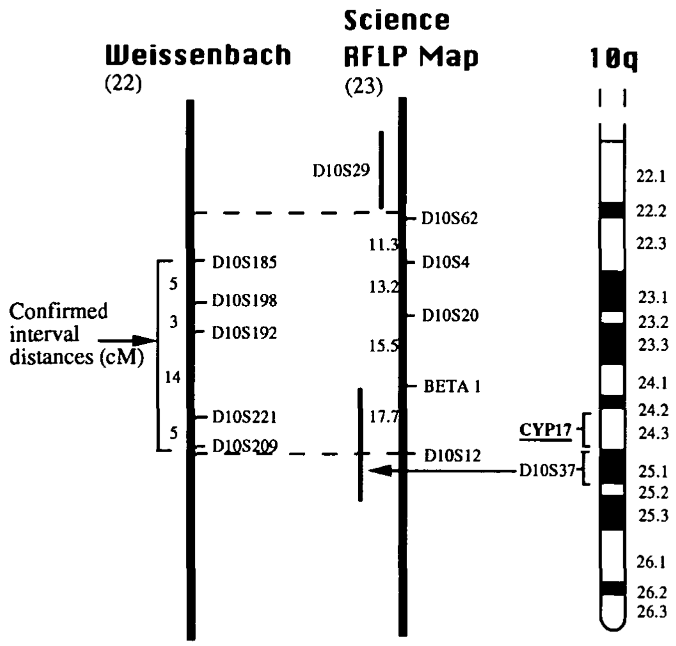

Figure 2. Chromosomal maps of 10q identifying marker loci and their confirmed interval distances (cM).

DISCUSSION

We have discovered a common base pair change within the 5′ region of CYP17 that has allowed its study as candidate gene for PCO/MPB within our families. We have demonstrated an association between those with the A2 allele of CYP17 and PCO in a group of consecutively identified affected women and a closely matched control group. The relative risk of a carrier of

Table 2. Pairwise lod scores between the disease locus and allele A2 of CYP17

	Lod Scores At Recombination Fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
	C/A	C/A	C/A	C/A	C/A	C/A	C/A
A2 Allele	−0.202/−0.237	−0.146/−0.208	−0.116/−0.016	−0.119/−0.112	−0.167/−0.18	−0.130/−0.142	−0.069/−0.074

C = Lod scores for Caucasian pedigrees only.
A = Lod scores for all pedigrees of all ethnic orgins.

Table 3. Pairwise lod scores between the disease locus and marker loci

	Lod Scores At Recombination Fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
	C/A	C/A	C/A	C/A	C/A	C/A	C/A
D10S185	−5.509/−6.207	−5.000/−5.635	−3.534/−2.638	−2.483/−1.154	−1.088/−1.154	−0.416/−0.416	−0.08/−0.075
D10S198	−1.639/−2.18	−0.867/−2.077	−0.681/−1.682	−0.502/−1.268	−0.252/−0.658	−0.103/−0.276	−0.02/−0.067
D10S192	−4.488/−5.429	−0.407/−4.943	−2.769/−3.406	−1.691/−2.106	−0.521/−0.672	−0.052/−0.052	−0.123/−0.125
D10S209	−7.672/−9.822	−6.977/−8.975	−5.280/−6.798	−3.947/−5.039	−2.157/−2.700	−1.035/−1.258	−0.354/−0.405
D10S221	−3.575/−5.145	−3.433/−4.87	−2.888/−3.94	−2.320/−3.056	−1.437/−1.79	−0.767/−0.909	−0.314/−0.346

this allele being affected is 3.57. This association is also seen when comparing our study group with a reference population of ethnically matched individuals whose affection status was unknown. The odds ratio is 2.50. This reduced relative risk reflects that approximately 15−20% of this group of controls will be affected.

Within the PCO/MPB pedigrees, association (but not linkage) between the affected state and the A2 allele was also observed. The prevalence of the A2 allele among unaffected individuals is 50% and the odds ratio was 2.2. The prevalence of the A2 allele in affected men in these families does not differ from that of affected women. The prevalence does not obviously differ between ethnic populations.

These data suggest that whilst CYP17 is associated with PCO/MPB, it cannot account for the underlying single gene aetiology for the disorder within these families. Because it does not consistently co-segregate with PCO/MPB and because members of the control populations with the A2 allele do not have PCO/MPB, CYP17 can be excluded as a causative gene for PCO/MPB, at least in most families.

What then is the functional significance of this association, and what model should we employ to analyse the genetics of polycystic ovary syndrome and male pattern baldness? The base pair change identified here creates a CCACC box recognition site in the 5′ transcribed, non-translated region of CYP17. There are four other such motifs within the 5′ region of this gene, three within the untranscribed promoter region and one immediately adjacent to the proposed initiation site of translation (underlined in the sequence data given) (10). It is thought that the number of 5′ promoter elements correlates with promoter activity (11) and it might therefore be expected that the creation of a further site may influence the promoter activity, thereby up-regulating transcription. This would be consistent with the hypothesis that the increased synthesis of androgens caused by the A2 allele of CYP17 may modify the clinical phenotype.

The segregation ratios observed from previous analysis of these pedigrees (1) suggest that there is one major dominant gene responsible for the expressed phenotype of PCO/MPB in each family. There may, however, be other genes (including CYP17)

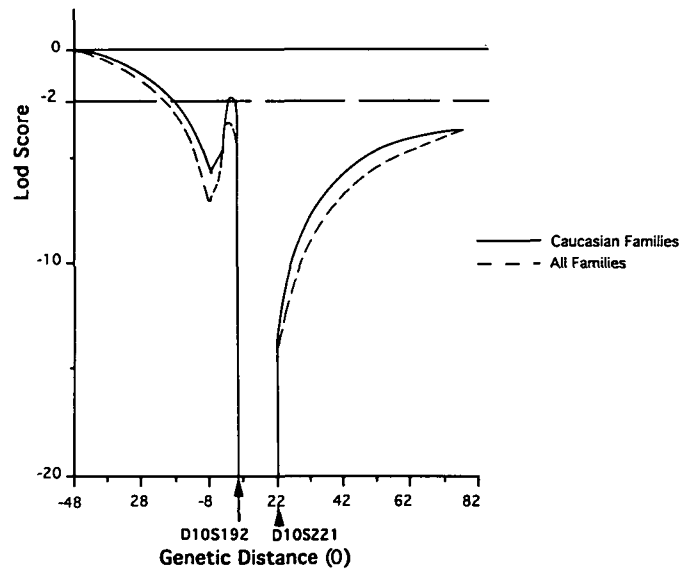

Figure 3. Location map summarising lod scores calculated for five loci in fourteen Caucasian families and twenty two families of varied ethnic background. The relative genetic position of D10S192 arbitrarily is put at 0.

which have a modifying activity on the expression of the phenotype.

If PCO/MPB is caused by another mutation at a separate locus which leads to an overproduction of androgens, then the presence of the A2 allele in CYP17 may further affect androgen production, altering the phenotype. If a woman has only the causative mutation she may demonstrate the ovarian morphology, but be asymptomatic or express a minimal clinical phenotype. However in the presence of the A2 allele this clinical phenotype may be more severe. This would explain the high degree of clinical and biochemical heterogeneity seen in patients presenting with polycystic ovary syndrome.

MATERIALS AND METHODS

Subjects

The fourteen Caucasian pedigrees ($n = 142$), of a total of twenty pedigrees ($n = 198$), were identified from 71 probands collected consecutively and seen at the Samaritan and St Mary's Hospital, London. Each proband presented with anovulation or hirsutism (or both) and was diagnosed as having polycystic ovaries (PCO) by pelvic ultrasound scan. The ultrasound scans were performed transabdominally by either AHC or DW and the ovarian morphology defined (2,12). Of the six non-Caucasian pedigrees identified and screened three were Asian, two Iranian, and one Afro-Caribbean. All family members underwent a full screening procedure; women of reproductive age were assigned as either normal or affected on the basis of the presence or absence of the typical ovarian morphology as determined by ultrasound. Assignment of affected postmenopausal women for linkage analysis was made on the basis of a positive history of hirsutism or menstrual disturbance, in accordance with our previously published data (1). All other women were assigned as unknown and excluded from the association analysis.

Men were considered to be positively affected if they demonstrated significant premature male pattern balding (MPB), defined as greater than a revised Hamilton IIa score (13), before the age of thirty years. Men over the age of 29 and without balding were considered unaffected but men without balding who were less than thirty years of age were excluded from further analysis.

A control group of 33 non-hirsute women with regular menses and identified as having normal ovarian morphology were recruited, of whom 24 were Caucasian. A larger reference group of 117 ethnically matched, unrelated individuals, whose status was unknown, were also screened for the presence of the CYP17 mutation. Genomic DNA was isolated from all family members and the two control groups.

Statistical analysis of frequency differences between groups was evaluated using a χ^2 or Fisher's exact test when necessary. Statistical significance was taken as $P<0.05$. Approval for this study was obtained from the ethics committee of Kensington Chelsea and Westminster Health Authority.

Identification of and screening for A2 allele

A PCR fragment of 459 bp containing the base pair change was generated using the following primers designed from the published sequence (10); Forward CATTCGCACTCTGGAGTC, Reverse AGGCTCTTGGGGTACTTG. Polymerase chain reaction (PCR) amplification of genomic DNA was performed using a Perkin Elmer-Cetus thermocycler. The 50 μl amplification mixture contained 50 ng of genomic DNA, 100 pmol of each primer, 1×Cambio reaction buffer, 100 μM each of dTTP/dCTP/dGTP/dATP and 1 unit of Amplitaq DNA polymerase (Cambio, UK). The reaction conditions were: 35 cycles of denaturation at 94°C (1 min), annealing at 57°C (1 min) and extension at 72°C (1 min). An initial denaturation step of 5 minutes at 95°C was employed with a final extension at 72°C for 10 minutes.

Heteroduplex analysis, on Mutation Detection Enhancement gels and sequencing of the PCR product was performed using standard methods (14–16).

Screening of the PCR product for the identified base pair change was by restriction digestion of the product with *Msp*A1 (NEB) and separation of the fragments by gel electrophoresis. A quantity of 0.2–0.5 units of enzyme per reaction, with 3 μl buffer (+BSA), were added directly to a 25 μl PCR product mixture to a final volume of 30 μl and incubated overnight at 37°C. To each sample, 5 μl of 6×gel loading buffer was added and 20 μl of this was seen in a 6% polyacrylamide non-denaturing electrophoresis gel. The gel was stained with ethidium bromide (100 μl of 10 mg/ml Sigma) in 1×TBE for 30 minutes and destained in 1×TBE for 10 minutes.

Linkage analysis

All members of these pedigrees were typed for five Genethon polymorphic microsatellite loci, D10S185, D10S198, D10S192, D10S221, D10S209, described elsewhere (17,18), from the chromosomal region 10q23.4. Genotype analysis was carried out following PCR amplification using a Perkin Elmer/Cetus thermocycler.

Linkage analyses were calculated using LINKAGE 5.1 (19) using a disease frequency of 15%, calculated from the mean of two previous population studies that had determined the incidence of PCO (20) and MPB(21). The penetrance was taken to be 0.95 (1). In the absence of any sex differences, lod scores were computed for combined sexes ($\{\theta_{male} = \theta_{female}\}$). No allowance for interference was made in the multipoint calculations.

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