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ARTICLE

Relationship between E23K (an established type II diabetes-susceptibility variant within *KCNJ11*), polycystic ovary syndrome and androgen levels

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Polycystic ovary syndrome (PCOS) is strongly associated with hyperinsulinaemia and type II diabetes (T2D). Sequence variation within *KCNJ11* (encoding Kir6.2, the beta- cell inwardly rectifying potassium channel) is implicated in the pathogenesis of neonatal diabetes, hyperinsulinaemia of infancy and multifactorial T2D. Comprehensive tagging studies have demonstrated that the *KCNJ11* E23K variant (or *ABCC8* A1369S in LD > 0.9) is responsible for the known association between *KCNJ11* and T2D. Given the phenotypic overlap between PCOS and T2D, we investigated whether E23K is involved in susceptibility to PCOS and related traits. Case–control analyses for the *KCNJ11* E23K variant were performed in (a) 374 PCOS cases and 2574 controls of UK British/Irish origin, and (b) 550 women with PCOS symptoms and 1114 controls from a Finnish birth cohort. The relationship between E23K genotype and androgen levels (a key intermediate phenotype relevant to PCOS) in 1380 samples was studied. The UK case–control analysis revealed no association between E23K genotypes and PCOS status ($P = 0.49$; Cochran–Armitage test), and no significant relationship between E23K genotype and androgen measures in the samples for which these phenotypes were available ($P = 0.19$). Similarly, the Finnish case–control analysis showed no association between E23K genotypes and PCOS status ($P = 0.75$; Cochran–Armitage test), and no significant relationship between E23K genotype and androgen measures in the samples for which these phenotypes were available (Finnish controls, $P = 0.25$; Finnish cases, $P = 0.08$). In conclusion, these data (involving >4600 subjects) provide no evidence that common variants of the *KCNJ11* E23K polymorphism have a major influence on PCOS susceptibility, though modest effect sizes (OR < 1.25) cannot be excluded.
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Introduction

Polycystic ovary syndrome (PCOS) is the commonest female endocrinopathy, affecting up to 10% of premenopausal women.[1] As well as the characteristic endocrine and reproductive features, women with PCOS frequently display profound metabolic disturbances. The risk of type II diabetes (T2D) in women with PCOS is approximately twice that of age- and BMI-matched controls.[2]

Although the aetiology of PCOS remains poorly understood, hyperinsulinaemia clearly plays an important role. Measures that reduce insulin resistance and hyperinsulinaemia improve both the metabolic and reproductive features of PCOS.[3–5] The adverse effects of hyperinsulinaemia are mediated via actions in numerous tissues including ovary, adrenal,[6–8] pituitary[9,10] and liver (through suppressed synthesis of sex hormone-binding globulin (SHBG)).[11,12]

Despite compelling evidence that PCOS has a significant heritable component, the variants responsible have yet to be defined,[6] although there is promising evidence of a susceptibility effect on chromosome 19p13.2.[13] Given the central role of insulin to PCOS pathogenesis, those genes whose products influence either the secretion or action of insulin represent important potential candidates.

One such gene (*KCNJ11*) encodes the inwardly rectifying potassium channel (Kir6.2), an essential component of the beta-cell ATP-sensitive potassium (K_{ATP}) channel. Rare mutations in *KCNJ11*[14] can lead to neonatal diabetes and hyperinsulinaemia of infancy (depending on the direction of their effect on channel function).[15] Previous comprehensive tagging studies have demonstrated that common variation within the single exon of *KCNJ11*, in particular the E23K variant (which is in almost complete LD (>0.9) with the A1369S variant within the neighbouring *ABCC8* but with no other nonsynonymous variants in *KCNJ11*), is heavily implicated in susceptibility to multifactorial T2D.[16] Furthermore, E23K is the polymorphism within *KCNJ11* most likely to be aetiological for T2D, on both statistical and functional grounds.[16–19] Given the strong metabolic, physiological and epidemiological overlap between PCOS and T2D (thereby obviating the need for retagging of *KCNJ11* in PCOS), we set out to test the hypothesis that the E23K polymorphism might impact on PCOS risk, through case–control studies and analysis of androgen levels as a continuous phenotype relevant to PCOS.

Materials and methods

UK case–control analysis

All of the UK cases have PCOS as defined by the 2003 Rotterdam consensus.[20] The cases were recruited as described previously.[21] All had presented with menstrual disturbances (oligo- or amenorrhoea defined as an intermenstrual interval >42 days) and/or hyperandrogenism and had ultrasound-confirmed polycystic ovarian (PCO) morphology.[22] Hyperandrogenism was defined clinically (hirsutism or acne) and/or biochemically (serum total testosterone >2.7 nmol/l). Other potential endocrine and neoplastic causes of hyperandrogenaemia were excluded.[20] A total of 374 nonpregnant PCOS cases from the UK (all of European British/Irish origin) were available. Two UK control groups were used for comparison. The first included 550 UK subjects ('HRC+'), 480 of which constitute the Human Random Control Resource (HRC) plus 70 additional samples from the same source (European Collection of Cell Cultures, Salisbury, UK). No phenotypic data are available for the HRC controls, although gender has been defined (270 are female) using an in-house SRY genotyping assay (details available on request). The second comprised 2024 members (1010 female) of the 1958 British Birth Cohort (58BC), followed longitudinally since birth during a single week in March 1958. Access to the 58BC samples was restricted to their use as controls and did not extend to availability of associated phenotype data. All controls are of UK European origin. As these were population controls (and as there was no evidence of a male *vs* female difference in genotype frequencies), cases were compared with all controls to maximise the precision of genotype frequency estimates (though we also present comparisons with female controls).

Finnish birth cohort

We also studied 1664 women from the Northern Finland Birth Cohort of 1966 (NFBC66).[23] These were selected from a total of 3115 cohort women who were examined at age 31 years and who consented for research use of their data. Those selected included 550 women with symptoms of PCOS (hirsutism and/or oligo-amenorrhoea defined as 'an intermenstrual interval >35 days') at age 31 years and 1114 control women (who reported no such symptoms). The former group ('symptomatic cases') have biochemical features – as a group – consistent with PCOS.[24,25] Because these cases do not reach the 2003 Rotterdam consensus definition of PCOS[20] (though as a group they have an increased prevalence of ultrasound-confirmed PCO morphology compared with controls),[26] we label these as 'symptomatic cases'. Available phenotypic data include BMI, serum testosterone and SHBG.

Clinical features are provided in Table 1. Serum testosterone and SHBG concentrations in the UK and Finnish groups were measured as described previously.[21] Free androgen index (FAI) was calculated as total testosterone $\times 100$/SHBG. All clinical investigations were conducted in accordance with the guidelines in the Declaration of Helsinki, and the study was approved by the relevant ethics committees in the United Kingdom and Finland. All subjects provided fully informed consent.

Genotyping

In UK cases and HRC+controls, the E23K variant (rs5219:C>T) was typed using a PCR-restriction fragment

length polymorphism (RFLP) method as described previously.[27] Other E23K genotyping was performed by Kbiosciences (www.kbioscience.co.uk: Hoddesdon, UK) using a Taqman Assay-on-Demand assay (Applied Biosciences, Warrington, UK) in NFBC66, and a fluorescence-based competitive allele-specific (KASPar) assay in 58BC. Details of all assays are available on request. For the E23K analysis in the Finnish symptomatic cases group, genotype success rate exceeded 93%. For the E23K analyses in all other groups, genotype success rates exceeded 95%. Based on a total of 1070 duplicate samples, the discrepancy error rate was estimated at 0.2%. There were no departures from Hardy–Weinberg equilibrium ($P>0.05$).

Statistical analyses

The Cochran–Armitage (additive) test was used for genotype-based case–control analyses (StatXact v.6., Cytel Corp., Cambridge, MA, USA). Quantitative trait analyses within UK cases and Finnish samples were conducted in SPSS (v12.0; SPSS Inc., Chicago, IL, USA) by one-way ANOVA following appropriate distributional transformations. Testosterone levels were optionally adjusted for BMI. Because no appreciable gender differences in genotype frequencies were observed, the main UK analyses included all control samples, to maximise the precision of estimates of population genotype frequencies. While use of population-based controls (rather than subjects in whom PCOS has been directly excluded) causes some loss of power, this is generally modest and can readily be overcome (as here) by increasing the sample size.[28]

Power calculations

Power calculations were made under a log-additive model using Quanto v.0.5.5. In both UK and Finnish case–control analyses, available sample sizes provided >80% power to detect an allelic OR at E23K >1.25 (and >40% power for OR >1.15) given an $\alpha=0.05$. In the largest of the samples used for the continuous trait analyses, we had >80% power to detect a between-genotype trait difference exceeding 22% of the SD ($\alpha=0.05$).

Results

UK case–control sample

In the absence of significant genotype frequency differences at E23K between the two UK control subgroups, or between genders, the principal case–control analysis compared UK cases against the entire control group (Table 2). The minor (K) allele frequency in PCOS cases (34.6%) and controls (35.9%) was similar and genotype frequency comparisons confirmed no association (Cochran–Armitage test, OR (per K allele) = 0.95 (95% CI = 0.80–1.11), $P=0.49$; recessive model KK *vs* E carriers, OR = 1.00 (0.72–1.39), $P=0.99$). Comparison of cases with female controls alone was also nonsignificant (OR = 0.94 (0.79–1.12), $P=0.50$; recessive model KK *vs* E carriers, OR = 0.90 (0.64–1.27), $P=0.55$). The K allele frequency in these UK controls is almost identical to that reported in other UK samples.[17]

Finnish case–control sample

The K allele frequency in the Finnish controls, although higher than that in the UK controls (47.6 *vs* 35.9%, respectively), is almost identical to that reported in other Finnish samples.[29] There was no association between E23K genotypes and case–control status in the Finnish cohort (Cochran–Armitage test, OR = 0.98 (0.84–1.13), $P=0.75$, Table 2; recessive model KK *vs* E carriers, OR = 0.93 (0.72–1.19), $P=0.56$).

Androgen levels

Analyses were separately conducted in UK cases ($n=156$), Finnish cases ($n=284$) and Finnish controls ($n=940$), after excluding those taking hormonal therapy, metformin or

Table 1 Clinical characteristics of UK and Finnish subjects

	UK PCOS cases	*UK HRC+ controls*	*UK BC58 controls*	*Finnish symptomatic cases*	*Finnish controls*
Number	374[a]	550	2024	550	1114
Female (%)	100	49.0	49.9	100	100
Age	32.0 [7.1][b]	Not known	Not available	31[c]	31[c]
BMI (kg/m^{-2})	26.9 (20.7, 35.0)	Not known	Not available	24.5 (20.2, 29.7)	23.8 (20.0, 28.4)
Waist-to-hip ratio (WHR)	0.79 (0.72, 0.87)	Not known	Not available	0.82 (0.75, 0.90)	0.81 (0.74, 0.88)
Testosterone (nmol/l)	2.08 (1.42, 3.02)[d]	Not known	Not available	2.01 (1.21, 3.02)[e]	1.80 (1.21, 2.77)[e]
Free androgen index (FAI)	5.20 (2.43, 11.13)[d]	Not known	Not available	3.99 (2.15, 7.38)[e]	3.09 (1.63, 5.76)[e]
Glucose (mmol/l)	4.8 (4.3, 5.3)[d,f]	Not known	Not available	4.9 (4.4, 5.5)[e,f]	4.9 (4.3, 5.6)[e,f]

Quantitative data are presented as geometric mean (SD range) unless otherwise stated.
[a]UK nonpregnant PCOS cases.
[b]Data shown as mean [SD].
[c]All women in the NFBC were sampled at the age of 31.
[d]Excluding those women on oral hypoglycaemic agents, metformin or hormonal therapy (oral contraception).
[e]Excluding those women on oral hypoglycaemic agents, metformin, hormonal therapy (oral contraception or hormonal intrauterine device) or those women pregnant at the time of examination.
[f]Fasting samples.

Table 2 Case–control association analyses for the relationship between the E23K variant (rs5219:C > T) of *KCNJ11* and PCOS in UK and Finnish groups

	Cases						*Controls*				
Alleles	*EE*	*EK*	*KK*	*Total*			*EE*	*EK*	*KK*	*Total*	P-*value vs cases*
UK cases	162 (43.9%)	159 (43.1%)	48 (13.0%)	369	UK HRC+	All	217 (41.0%)	238 (45.0%)	74 (14.0%)	529	0.41[a]
						Females only	115 (44.4%)	110 (42.5%)	34 (13.1%)	259	0.95[a]
					UK58BC	All	816 (41.4%)	906 (45.9%)	251 (12.7%)	1973	0.56[a]
						Females only	412 (41.8%)	430 (43.7%)	143 (14.5%)	985	0.40[a]
					Combined	All	1033 (41.3%)	1144 (45.7%)	325 (13.0%)	2502	0.49[a]
						Females only	527 (42.4%)	540 (43.4%)	177 (14.2%)	1244	0.50[a]
Finnish symptomatic cases	146 (28.4%)	253 (49.2%)	115 (22.4%)	514	Finnish controls	Females only	302 (28.5%)	506 (47.8%)	251 (23.7%)	1059	0.75[b]

Data shown are genotype counts (and percentages). *P*-values represent Cochran–Armitage test results.
[a]Comparison with UK PCOS cases.
[b]Comparison with Finnish symptomatic cases.

Table 3 *KCNJ11* E23K genotypes and analyses of androgen measures

		EE	*EK*	*KK*	*Total*	P-*value*	P-*value adjusted for BMI*
UK cases	*n*[a]	68	71	17	156		
	Testosterone (nmol/l)	2.0 (1.3, 3.0)	2.2 (1.6, 3.1)	2.0 (1.3, 3.0)		0.19	0.15
	FAI	5.1 (2.3, 11.3)	5.7 (2.7, 11.9)	3.5 (2.0, 6.2)		0.16	
Finnish controls	*n*[b]	266	446	228	940		
	Testosterone (nmol/l)	1.8 (1.2, 2.8)	1.9 (1.2, 2.8)	1.8 (1.2, 2.7)		0.25	0.12
	FAI	3.1 (1.6, 6.1)	3.1 (1.7, 5.8)	3.0 (1.7, 5.6)		0.75	
Finnish symptomatic cases	*n*[b]	83	131	70	284		
	Testosterone (nmol/l)	2.0 (1.4, 2.9)	2.0 (1.4, 2.9)	2.2 (1.5, 3.4)		0.08	0.30
	FAI	3.7 (2.1, 6.6)	4.1 (2.2, 7.6)	4.3 (2.4, 8.0)		0.11	

Testosterone and FAI values are expressed as geometric mean (SD range); FAI = free androgen index (total testosterone/SHBG × 100).
[a]All UK PCOS British/Irish cases excluding those women on oral hypoglycaemic agents (including metformin) or hormonal therapy.
[b]Excluding those women on oral hypoglycaemic agents (including metformin), hormonal therapy or those women pregnant at the time of examination.
FAI was not adjusted for BMI given the high correlation between BMI and SHBG concentration.

oral hypoglycaemic agents (and after exclusion of those women pregnant at the time of examination in the Finnish groups). There were no significant differences in testosterone concentrations or FAI with respect to E23K genotype in any of the groups (Table 3), nor was there any consistent pattern or trend across the three groups.

Discussion

This is the first study to analyse the relationship between variation at the *KCNJ11* E23K polymorphism and PCOS. In case–control and quantitative trait analyses, we found no evidence that this variant was associated with the development of PCOS, or related to relevant quantitative traits (principally, androgen measures).

Because there are over 20 000 genes in the human genome, the biological significance of this finding depends in large part on the credibility of the E23K variant within *KCNJ11* as a candidate for PCOS. The E23K variant is established as one of the few polymorphisms with a convincingly replicated impact on T2D susceptibility. *In vitro*[19] and *in vivo*[30] functional studies indicate that the K allele at E23K is associated with an increased open probability of the beta-cell K_{ATP} channel and reduced insulin secretion.[19]

In this respect, the strong clinical, phenotypic and epidemiological overlap between T2D and PCOS promotes a powerful argument for overlapping genetic influences. However, the relationships between PCOS, T2D and insulin levels are complex. Although, as with T2D, PCOS is characterised by peripheral insulin resistance, a wide range of abnormalities of beta-cell function have also been reported. These include both reduced beta-cell function,[31–34] and increased acute insulin secretion from the beta cell during an oral glucose tolerance test.[35,4,36] These

data are most plausibly reconciled with the known effects of excess insulin action within the ovary, through a model that invokes differential insulin sensitivity between tissues. This provides a mechanism whereby the ovary is subjected to excessive insulin action despite peripheral insulin resistance and appropriate, but inadequate, compensatory hyperinsulinaemia. Under such a model, a variant such as E23K that influences beta-cell function might, in principle, be capable both of increasing the risk of T2D and reducing that of PCOS. The present study would, of course, have been able to detect powerful associations in either direction. In the absence of a significant association with PCOS, these genetic data do not allow us to distinguish between these alternative mechanisms.

It is important to realise, however, that while the present study involves data on over 4600 individuals, the available sample sizes place inevitable limitations on interpretation of these findings. The numbers in either the UK or Finnish case–control subsets were sufficient to detect an OR >1.25 (with $\alpha=0.05$ and 80% power). Although this OR threshold roughly equates to the effect size of E23K on susceptibility to T2D seen in early meta-analyses,[17] more recent meta-analyses have suggested that the effect of E23K on T2D susceptibility is lower (OR$\sim$1.15).[16,37] The power of the present study to detect effect sizes with OR<1.25 is limited. Furthermore, ovarian ultrasound data were not available for most of the Finnish symptomatic cases. Therefore, the possibility of false negative results in the Finnish analyses cannot be excluded.

In summary, our study provides no evidence that variation at the *KCNJ11* E23K polymorphism, known to be associated with diabetes risk, influences PCOS susceptibility or levels of androgens. These data argue against a direct overlap in genetic susceptibility between T2D and PCOS. However, analysis of samples several times larger than those available in this study (or most other current studies of PCOS genetics for that matter) would be required to exclude variation at the *KCNJ11* E23K polymorphism from a more modest role in disease susceptibility.

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