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SHORT COMMUNICATION

Disparate genetic influences on polycystic ovary syndrome (PCOS) and type 2 diabetes revealed by a lack of association between common variants within the *TCF7L2* gene and PCOS

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Abstract
Aims/hypothesis Common variants of the gene encoding transcription factor 7-like 2 (*TCF7L2*) have a powerful effect on individual risk of type 2 diabetes (per allele odds ratio ~1.35). Polycystic ovary syndrome (PCOS) and type 2 diabetes are familial conditions sharing common features. Based on this, the aim of the present study was to establish whether variation in *TCF7L2* also influences the development of PCOS.
Methods We conducted a genetic association study of variants of *TCF7L2* (rs7903146 and rs12255372) using both case–control and quantitative trait approaches. Case–control analyses were conducted in (1) 369 PCOS cases and 2574 controls of UK British/Irish origin, and (2) 540 women with PCOS symptoms and 1083 controls from the Northern Finland Birth Cohort of 1966. Quantitative trait analyses (androgen levels) were also performed (1249 individuals).
Results There was no association between rs7903146 and PCOS in the UK case–control study (Cochran–Armitage test, $p=0.51$); nor with symptomatic status in the Finnish cohort ($p=0.36$). In addition, there were no relationships between the *TCF7L2* single nucleotide polymorphism rs7903146 and androgen levels (UK cases, $p=0.99$; Finnish controls, $p=0.57$; Finnish symptomatic cases, $p=0.80$). Results at rs12255372 were similar, reflecting strong linkage disequilibrium with rs7903146.

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Conclusions/interpretation Our study was powered to detect an effect on PCOS susceptibility similar to that previously reported for these variants on type 2 diabetes. Failure to detect any evident association with PCOS provides the strongest evidence yet that the genetic architecture of these related conditions is qualitatively distinct.

Keywords Polycystic ovary syndrome · *TCF7L2* variants · Type 2 diabetes mellitus

Abbreviations

58BC	1958 British Birth Cohort
FAI	Free androgen index
HRC+	Human Random Control
NFBC1966	Northern Finland Birth Cohort of 1966
OR	Odds ratio
PCOS	Polycystic ovary syndrome
SHBG	Sex hormone-binding globulin
SNP	Single nucleotide polymorphism
TCF7L2	Transcription factor 7-like 2

Introduction

Polycystic ovary syndrome (PCOS) and type 2 diabetes mellitus share common features. These include pathophysiology (abdominal obesity, insulin resistance and disturbed beta cell function are characteristic of both conditions) and epidemiology (many women with PCOS also develop type 2 diabetes, and vice versa) [1]. Given compelling evidence for a substantial genetic contribution to susceptibility to both conditions [1], known type 2 diabetes susceptibility genes emerge as strong candidates for a role in the development of PCOS.

Common variants in the gene encoding transcription factor 7-like 2 (*TCF7L2*) have been reproducibly shown to display powerful associations with type 2 diabetes in a number of studies [2–4], with a typical per allele odds ratio (OR) of ~1.35. Most current evidence favours impaired insulin secretion as the mechanism responsible [3, 5]. Although insulin resistance clearly plays a major role in the aetiology of PCOS, there is evidence for a concomitant (potentially primary) disturbance of beta cell function [6], endorsing the biological candidacy of *TCF7L2* with respect to susceptibility to PCOS.

The causal variant within *TCF7L2* has yet to be definitively identified. However, two single nucleotide polymorphisms (SNPs; rs7903146 and rs12255372) within *TCF7L2* display the strongest effects on type 2 diabetes susceptibility in European populations [2], with rs7903146 the better marker overall [7]. We tested the hypothesis that these *TCF7L2* SNPs denoting type 2 diabetes risk also influence susceptibility to PCOS, using both case–control studies and analysis of androgen levels as a continuous trait relevant to PCOS.

Methods

UK case–control analysis All 369 UK cases had a definitive diagnosis of PCOS according to the Rotterdam diagnostic criteria [8]. Recruitment details have been described elsewhere [9]. All had a history of oligo- or amenorrhoea (inter-menstrual interval >42 days) and/or hyperandrogenism, the latter defined clinically (hirsutism or acne) and/or biochemically (serum total testosterone >2.7 nmol/l). In addition, all cases had polycystic ovarian (PCO) morphology confirmed on ultrasound [8]. All other potential endocrine and neoplastic causes of hyperandrogenaemia were excluded prior to recruitment. All were of European British/Irish origin and not pregnant at the time of study.

For comparison, controls from two population-based UK samples (all of European British/Irish origin) were used, as previously described [9]. The Human Random Control and 1958 British Birth Cohort control groups comprise 550 (270 women) and 2024 individuals (1010 women), respectively. Additional phenotypic data are not available for these controls. *TCF7L2* genotype data for both control groups have previously been reported [4]. As there are no significant differences in genotype frequencies between the two UK control groups, our primary case–control analyses compared UK cases with the entire control group. In addition, since these are population-based controls and there is no evidence of sex-specific differences in genotype frequency, our primary analysis included controls of both sexes, though we also present analyses restricted to female controls. Use of population-based controls results in some loss of power compared with controls in whom disease has been excluded, but this is modest and easily overcome (as here) by increasing the control sample size.

Northern Finland Birth Cohort of 1966 In recognition of the spectrum of PCOS-related phenotypes within the general female population, we also analysed genotypes from 1623 singleton women from the Northern Finland Birth Cohort of 1966 (NFBC1966). This sample comprised 540 women with symptoms of PCOS (hirsutism and/or oligo- or amenorrhoea, defined as an inter-menstrual interval of >35 days), and 1083 controls (a random sample of women who reported no such symptoms), all examined at age 31 years [10]. A definitive diagnosis of PCOS [8] is not possible in this cohort; however, compared with the controls, these ‘symptomatic cases’ have an increased

Table 1 Clinical characteristics of UK and Finnish NFBC1966 subjects

	UK PCOS cases	UK HRC+ controls	UK 58BC controls	Finnish symptomatic cases	Finnish controls
n	369[a]	550	2024	540	1083
Women (%)	100	49.0	49.9	100	100
Age (years)	32.0±7.1	Not known	Not available	31[b]	31[b]
BMI (kg/m^2)	26.9 (20.7–34.9)	Not known	Not available	24.4 (20.3–29.4)	23.8 (20.0–28.3)
Waist to hip ratio	0.79 (0.72–0.88)	Not known	Not available	0.82 (0.74–0.90)	0.81 (0.73–0.88)
Testosterone (nmol/l)	2.1[c] (1.4–3.3)	Not known	Not available	2.0[d] (1.4–3.0)	1.8[d] (1.2–2.8)
FAI	5.5[c] (2.4–11.8)	Not known	Not available	4.2[d] (2.1–7.3)	3.1[d] (1.7–5.9)
Glucose (mmol/l)	4.8[c,e] (4.3–5.3)	Not known	Not available	4.9[d,e] (4.5–5.4)	4.9[d,e] (4.3–5.6)

Quantitative data are presented as geometric means (SD range) or means ± SD
HRC+ = Human Random Control; 58BC=1958 British Birth Cohort
[a] UK non-pregnant PCOS cases
[b] All women in the NFBC1966 were sampled at the age of 31 years
[c] Excluding those women on oral hypoglycaemic agents, metformin or hormonal therapy (oral contraception)
[d] Excluding those women on oral hypoglycaemic agents, metformin, hormonal therapy (oral contraception or hormonal intra-uterine device) or those women pregnant at the time of examination
[e] Fasting samples

prevalence of ultrasound-confirmed PCO morphology and biochemical features consistent with PCOS [10].

The clinical features of all case and control groups are shown in Table 1. Serum testosterone and sex hormone-binding globulin (SHBG) concentrations in the UK and Finnish groups were measured as previously described [9]. The free androgen index (FAI) was calculated as [total testosterone×100]/SHBG. All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki as revised in 2000. All subjects provided fully informed written consent and the study was approved by the relevant ethics committees in the UK and Finland.

Genotyping In both the UK and Finnish samples, genotyping of rs7903146 and rs12255372 was performed by Kbiosciences (Hoddesdon, UK) using a fluorescence-based competitive allele-specific (KASPar) assay (details on request). Genotype success rates exceeded 95% in both the UK and Finnish samples. Based on 466 duplicate samples, the discrepancy error rate was estimated at <0.2% for both variants. There was no departure from Hardy–Weinberg equilibrium ($p>0.05$).

Statistical analyses and power calculations The Cochran–Armitage (additive) test was used for genotype-based case-

Table 2 Case–control association analyses for the relationship between variants at rs7903146 and PCOS in UK and Finnish groups

Genotype	Cases				Controls				*p* value vs cases
	CC	CT	TT			CC	CT	TT	
UK cases (*n*=358)	177 (49.4%)	151 (42.2%)	30 (8.4%)	UK HRC+	All (*n*=510)	243 (47.7%)	217 (42.5%)	50 (9.8%)	0.47[a]
					Females only (*n*=249)	117 (47.0%)	108 (43.4%)	24 (9.6%)	0.49[a]
				UK 58BC	All (*n*=1966)	932 (47.4%)	867 (44.1%)	167 (8.5%)	0.56[a]
					Females only (*n*=994)	483 (48.6%)	431 (43.4%)	80 (8.0%)	0.89[a]
				Combined	All (*n*=2476)	1175 (47.4%)	1084 (43.8%)	217 (8.8%)	0.51[a]
					Females only (*n*=1243)	600 (48.3%)	539 (43.4%)	104 (8.3%)	0.76[a]
Finnish symptomatic cases (*n*=476)	301 (63.2%)	156 (32.8%)	19 (4.0%)	Finnish controls	Females only (*n*=936)	620 (66.2%)	278 (29.7%)	38 (4.1%)	0.36[b]

Data shown are genotype counts (percentages) (reduced numbers reflect genotype success rates outlined in methodology). The *p* values represent Cochran–Armitage test results
HRC+ = Human Random Control; 58BC = 1958 British Birth Cohort
[a] Comparison with UK PCOS cases
[b] Comparison with Finnish symptomatic cases

control analyses (StatXact, version 6; Cytel, Cambridge, MA, USA). Quantitative trait analyses were conducted in SPSS (version 12.0; SPSS, Chicago, IL, USA), by one-way ANOVA following appropriate distributional transformations. Testosterone levels were optionally adjusted for BMI.

Power calculations were performed using Quanto, version 0.5.5 (log-additive model). In the UK and Finnish case–control analyses, sample sizes provided 85% and 70% power, respectively, to detect an allelic OR of 1.3 (rs7903146; α=0.05). The continuous trait analyses had 80% power to detect a between-genotype trait difference exceeding 93, 95 and 56% of a SD in the UK, Finnish symptomatic cases and Finnish control groups, respectively (α=0.05).

In all samples studied, and in accordance with the previous literature, the correlation between genotypes at rs7903146 and rs12255372 was high (r^2=0.79, 0.71 and 0.68 in UK female controls, UK cases and the Finnish cohort, respectively). Given evidence favouring rs7903146 (or a close proxy thereof) as the aetiological variant, we present the data focusing on this SNP.

Results

The minor allele frequency for rs7903146 in UK PCOS cases was 29.5%, similar to that seen in the UK control groups (30.7%). Genotype frequency comparisons revealed no association with PCOS (Cochran–Armitage test, OR [per minor allele] 0.95, 95% CI 0.80–1.12, p=0.51; Table 2). Comparison with female controls alone was also non-significant (OR 0.97, 95% CI 0.81–1.17, p=0.76). In the Finnish cohort, too, there was no association between rs7903146 and case–control status (Cochran–Armitage test, OR [per minor allele] 1.10, 95% CI 0.90–1.34, p=0.36; Table 2). Following the exclusion of women taking hormonal therapy, metformin or other oral hypoglycaemic agents, continuous trait analyses were conducted separately in UK cases (n=168), Finnish symptomatic cases (n=259) and Finnish controls (n=822). Neither testosterone levels nor FAI showed a relationship with rs7903146 genotype in any of the groups (testosterone: UK cases, p=0.99; Finnish controls, p=0.57; Finnish symptomatic cases, p=0.80). Analyses of rs12255372 generated similar results throughout (data not shown).

Discussion

This is the first study to test the hypothesis that type 2 diabetes-associated variants of *TCF7L2* also affect susceptibility to PCOS. We found no evidence that these variants are associated with the development of PCOS or PCO-related phenotypes in well-powered case–control and quantitative trait analyses.

Common variants of *TCF7L2* most likely influence type 2 diabetes susceptibility through impairment of insulin secretion [3]. The largest study to date, which genotyped *TCF7L2* rs7903146 in 24,053 subjects including 4578 subjects from NFBC1966, has demonstrated a modest effect of genotype on the early insulin response to oral glucose [5]. Our findings therefore support the notion that, in contrast to type 2 diabetes, genetic variation influencing beta cell dysfunction is not a primary and essential determinant of PCOS pathogenesis. The present study was sufficiently powered to detect an effect size for PCOS similar in magnitude to that previously shown for type 2 diabetes susceptibility in UK populations (OR 1.36) [4]. Exclusion of a more modest effect than this would have required an appropriately larger sample.

To summarise, we provide evidence that common type 2 diabetes susceptibility variants of *TCF7L2* have no association with the development of PCOS and no detectable influence on androgen levels in women. Our data provide the strongest indication yet that, despite apparent epidemiological and pathophysiological similarities, PCOS and type 2 diabetes feature qualitatively distinct genetic susceptibility effects. Our study is consistent with a model whereby genetic and environmental factors combine to influence insulin resistance in both type 2 diabetes and PCOS. Whilst variants that affect the capacity of the beta cell to respond to insulin insensitivity are likely to predominate in defining individual risk of type 2 diabetes, we hypothesise that factors involved in the ovarian response to insulin resistance are likely to be key to the development of PCOS.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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