Genetic risk for the polycystic ovary syndrome, bone mineral density and fractures in women and men: A UK Biobank Mendelian randomisation Study

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Abstract

Introduction: There is conflicting data on the effect of polycystic ovary syndrome (PCOS) on bone mineral density (BMD) and fracture risk. Recent genetic data suggest that men may also carry genetic risk factors for PCOS; the associations of these factors with parameters of bone health remains unknown.We aimed to investigate if the genetic risk of PCOS is associated with BMD and fracture risk in women and men in the UK Biobank dataset.

Methods: We used Mendelian randomisation (MR) analysis to test the association of genetic risk of excess testosterone in PCOS with BMD and fractures in the UK biobank study. The MR analysis was performed using linear regression analysis with the weighted genetic risk score (wGRS) as an independent variable adjusting for age, BMI and population eigenvectors. The horizontal pleiotropy in the MR analysis was tested using MR-Egger regression analysis.

Results: The study consisted of 221,086 Caucasian women (mean age ± SD: 56.7 ± 7.9 years, mean body mass index [BMI] ± SD: 27.0 ±5.1 kg/m^2, mean BMD ± SD: 0.50 ±0.11 g/cm^2) and 187,816 Caucasian men (mean age ± SD: 57.1± 8.1 years, mean BMI ± SD: 27.7± 4.1 kg/m^2 and mean BMD ± SD: 0.56 ± 0.12 g/cm^2. Women and men self-reported 24,797 (11%) and 17,076 (10%) fractures over the last 5 years, respectively. The MR analysis showed that one SD increase in the wGRS for clinical or biochemical hyperandrogenism in PCOS was associated with significantly higher heel BMD (Beta= 0.0007 [±0.0002], P-value = 0.001) and a significantly reduced risk of fractures (OR=0.97, P-value = 0.003) in women. A similar wGRS in men was not associated with BMD or risk of fractures.

Conclusion: In this study, we show that the excess genetic risk for hyperandrogenism in women with PCOS is associated with a higher BMD and reduced risk of fractures.

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Running Title: PCOS and BMD

Introduction

Polycystic ovary syndrome (PCOS) is a common multisystemic disorder in women involving the endocrine, cardiovascular, and reproductive systems [1-3]. Specifically, PCOS is characterised by biochemical and clinical features of androgen excess (hirsutism and acne), menstrual irregularities and polycystic morphology of the ovaries [1-3]. PCOS is considered as a leading cause of anovulatory infertility in women of reproductive age group [3, 4].

There is conflicting data on the effect of PCOS on bone mineral density (BMD) and the risk of fractures in women with PCOS. It has been hypothesised that the excess testosterone in women with PCOS can positively influence the bones, directly through androgen receptors on bone-related cells or after conversion to 17β-estradiol and estrone [5]. Moreover, elevated circulating insulin levels seen in women associated with PCOS, may have anabolic effects on bone and offer some protection against bone loss in these women [5] .

Several population-based studies have shown a higher [6-10] BMD in the PCOS population; however, others have shown a lower BMD [11] or no association between PCOS and BMD [12]. A recent meta-analysis [13] of 21 studies showed that women with PCOS with a BMI of less than 27 kg/m2 had a reduced BMD, while women with PCOS with BMI more than 27 kg/m2 showed no such association between BMD and PCOS. These mixed results are likely due to confounding factors in observational studies, such as age, body mass index (BMI), various degrees of sex hormone disturbances, other endocrine disorders, and medication use, all of which influence the BMD [13].

It has been suggested that the primary defect in PCOS is not the disorder of ovaries itself but likely represents an endocrine and or metabolic disturbance and can affect men as well [14]. Recent data suggest the presence of cardiometabolic dysfunction in men with known genetic

risk factors for PCOS [15]. It is unclear if this excess genetic risk for PCOS affects BMD and risk of fracture in men.

Confounding factors in the observational studies can be largely overcome by using Mendelian randomisation (MR) studies [16, 17]. MR relies on genetic variants fixed at birth (eliminating reverse causation bias) to uncover causal relationships between exposure and outcome. Since the assortment of germline genetic variants during meiosis is random, MR is less likely to be influenced by environmental and lifestyle confounding variables.

In this study, we utilise a MR approach to test the association of excess genetic risk of clinical and biochemical hyperandrogenism in PCOS with BMD and risk of fractures in women and men in the UK Biobank cohort.

Methods

Study Population

UK Biobank is a prospective cohort of 502,635 participants (5.5% response rate) aged 40-69 years. All participants were recruited between 2006 and 2010 and attended one of the 22 assessment centres across UK, where they provided information on sociodemographic, lifestyle and health parameters and underwent physical and medical assessments. Blood samples were also collected for genotyping and biochemical analyses.

The UK Biobank protocol complied with the Declaration of Helsinki and was approved by the North West Multi-Centre Research Ethics Committee. Participants provided their informed consent on the touchscreen before taking part. The UK Biobank protocol is available online (http://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf)

Assessment of Bone Mineral Density and Fracture

In the UK Biobank, quantitative ultrasound of the heel was performed using the Sahara Clinical Sonometer (Hologic, Bedford, Massachusetts) with a standardised protocol. Fully trained staff checked if participants were able to undergo both left and right heel ultrasound measurement. The study participants with open wounds, breaks or sores around the heel, or metal parts in the heel did not undertake measurement of that heel. Each centre used the same machine model, and quality control was performed daily with a phantom according to the manufacturer's instructions. The mean values for BMDs (g/cm^2) and BMD T-scores for the left and right heel were computed. If the BMD data were available for both the left or right heel; we included the lower value of BMD in the analysis. History of fractures was assessed by self-reported data on fracture occurrence (yes or no) over the past five years.

Selection of GWAS SNPs and MR analysis

The GWAS SNPs for this study were obtained from the largest GWAS for PCOS thus far, with 10,074 PCOS cases and 103,164 controls of European ancestry [18].. *This analysis identified 3 novel loci (near PLGRKT, ZBTB16 and MAPRE1), and replicated of 11 previously reported loci with PCOS. These 14 common variants were also tested for their association with clinical and biochemical hyperandrogenism in women with PCOS and the effect estimates from these association were used to construct the genetic risk score in this present study.* Using the whole genome association analysis toolset, PLINK, the weighted genetic risk score (wGRS) was calculated by multiplying the estimated β-coefficient by the number of corresponding risk alleles. A linear regression analysis was used, in which BMD was the dependent variable and weighted genetic risk score (wGRS), age, BMI, and top five principal components (to adjust for population stratification) were independent variables.

Horizontal pleiotropy can cause bias in MR analysis, as it suggests that the variant (SNPs or genetic instrument) has an impact on the disease/outcome outside of its effect on the exposure.

To determine the presence of horizontal pleiotropy, we used the MR-Egger regression analysis. (MR-Egger). MR-Egger provides an estimate on whether the genetic variants associated with the risk factor are also directly associated with the outcome[19]. The intercept of the MR–Egger regression analysis indicates directional multiplicity [19] with a non-zero intercept indicating that the MR analysis is biased. The test of whether the intercept differs from zero is referred to as the MR-Egger intercept test. In the absence of horizontal pleiotropy, we reported the estimates from the wGRS regression analysis as it is more powered to detect the MR effect.

Power Calculation

Power calculation for the study was done using the online power calculation tool for the MR study (https://shiny.cnsgenomics.com/mRnd/). With an approximate 11% prevalence of fractures in the study and a 2% proportion of variance explained in the prevalence of fractures by the SNPs a sample size of 221,086 has more than 95% power to detect an ODDS ratio of 1.2. If the SNPs were to explain a 1% variance in the outcome, the study would have more than 75% power to detect the ODDS ratio of 1.2.

Results

The Mendelian randomisation model and the study hypothesis are shown in **Figure 1**. Using the UK Biobank data, we looked at the genetic risk for excess testosterone levels in PCOS with BMD. **Table 1** shows the demographic characteristics of the study population. The study consisted of 221,086 Caucasian women (mean age ± SD: 56.7 ± 7.9 years, mean body mass index [BMI] ± SD: 27.0 ±5.1 kg/m2, mean BMD ± SD: 0.50 ± 0.11 g/cm2) and 187,816 Caucasian men (mean age ± SD: 57.1± 8.1 years, mean BMI ± SD: 27.7± 4.1 kg/m2 and mean BMD ± SD: 0.56 ± 0.12 g/cm2. Study participants self-reported 24,797 (11%) all cause-fractures in females and 17,076(10%) in males over the last five years . **Supplementary Tables 1 and 2** show the association of the PCOS associated markers with BMD and fractures,

148 respectively. The top SNPs associated with BMD in women were rs11031005 (Beta=-0.0003,
149 P-value=1.88E-11) in or near *FSHB* gene, rs7563201(Beta=-0.001, P-value=0.0001) in or near
150 *THADA* gene and rs7864171 (Beta= -0.0001 ,P-value-0.0001) in or near the *FANCC* gene. The
151 top PCOS associated SNPs with fracture were rs1784692 (OR=1.03, P-value=0.01) on or near
152 the *ZBTB16* gene and rs9696009 (OR=0.93, P-value=0.95) on or near the *DENND1A* gene.
153 **Figures 2a and 2b** show scatter plots showing the associations of the PCOS associated SNP
154 effects on the testosterone levels against the SNP effects on the BMD and fractures,
155 respectively.

156 **Table 2** shows the association of the weighted genetic risk score for clinical and biochemical
157 hyperandrogenemia in PCOS with BMD and fracture in women and men in the UK Biobank.
158 The results show that one SD increase in the genetic risk for hyperandrogenism in PCOS was
159 associated with significantly higher BMD (Beta = 0.0007 [±0.0002], P-value 0.001) and a
160 significantly reduced risk of fractures (OR = 0.97 [0.96,0.99], P-value = 0.003) in women.

161 We did a sensitivity analysis using the effect estimates for PCOS rather than testosterone levels
162 in PCOS. The wGRS from this these effect estimates were also associated with a a trend
163 towards higher BMD (P-value = 0.07) and a significant reduction in fracture risk (OR = 0.98
164 [0.96,0.99], P-value = 0.007).

165 *In a further sensitivity analysis, we checked the association of the wGRS for hyperandrogenism*
166 *with BMI, an important factor that could affect the BMD. The genetic risk score for PCOS showed*
167 *no association with BMI in women (Beta =-0.001 SE= 0.004, P-value= 0.681) and men (Beta= -*
168 *1.72, SE= 1.02, P-value= 0.1) in the UKBiobank database. We also checked the association of the*
169 *individual SNPs used to obtain the wGRS with BMI and none were associated with BMI at*
170 *threshold of significance accepted for GWAS studies*

The MR analysis in men showed that one SD increase in the genetic risk for high testosterone levels in PCOS was not associated with BMD (P-value=0.43) or risk of fractures (P-value=0.47).

Genetic pleiotropy was tested using the significance levels for the intercept of the MR-Egger model. MR-Egger analysis showed a non-significant P-value for the intercept (P-value = 0.57). indicatingabsence of horizontal pleiotropy in the model and suggesting that the wGRS estimates are valid.

Discussion

In this large MR study of women and men, we showed that the excess genetic risk for clinical and biochemical hyperandrogenism in women with PCOS is associated with high heel BMD and reduced risk of fracture.

PCOS is considered an ancient disorder [20-22], and several ancient records (Hippocrates (460 BC - 377 BC) Ephesus (c. 98-138 AD)) have discussed the presence of PCOS-like characteristics in women [22]. PCOS is a leading cause of subfertility in women [23], and given its detrimental effect on fertility and a resulting negative selection, pressure should have reduced the prevalence of PCOS. However, PCOS continues to be an extremely common endocrine and metabolic disorder, giving rise to the PCOS paradox [22]. It has been suggested that some of the genetic susceptibilities for PCOS could have offered survival advantages in ancient environments [22]. Several observational studies showing higher BMD [7-10] and reduced risk of fractures [9] which could have contributed to improved fitness and survival

advantages in the ancient populations. The available observational studies on PCOS and BMD have often yielded contradictory results indicating increased ordecreased BMD in women with PCOS. For example, a prospective study with a 21-year follow-up [24] showed no differences in muscle mass, BMD and incident fracture between women with PCOS with persistently higher free androgen index (FAI) and controls.In contast, Zborowski et al. [7] observed a significantly higher mean BMD at five out of the six bone sites measured among older women with PCOS compared with control women of similar age without PCOS. Another Danish study [9] showed that women with PCOS were less likely to have all-cause fractures, major osteoporotic fractures and fractures of the head and face as compared to controls and the risk reduction was more pronounced in the younger age group. These conflicting results are likely due to confounding factors in PCOS, such as degree of insulin resistance, varying oestrogen levels and medications [1]. We addressed this by using an MR approach [16, 17] and showed that genetic predisposition to excess testosterone in PCOS is associated with a higher BMD and a reduced risk of fractures.

The association of estrogens on BMD inwomen has been examined extensively [5], however there is limited data on the effects of androgens on BMD in women[25]. In a study involving 30 Caucacian women, Buchanan et al. [26] first showed that the androgen and estrogen function as independent and additive determinants of peak trabecular bone density in young women. It has also been shown that some anti-androgen treatments can significantly reduced BMD in women with androgen excess [27]. Another study by C Slemenda[28] has shown that bone loss was significantly associated with lower androgen concentrations in premenopausal , perimanopatusal and post-menopausal women. These findings support the positive influence of androgens on BMD and support the findings of our MR analysis.

PCOS is predominantly considered a female endocrine, metabolic and reproductive disorder, however, some studies have explored the possibility of the male equivalent of PCOS [14, 29].

Recent data [15] from the UK Biobank shows that men with excess genetic risk for PCOS show 17% increased risk of obesity, 15% increased risk of type 2 diabetes and 5% increased risk of coronary artery disease. These data suggest that the genetic markers for PCOS can act independently of the ovaries. In the present study, we show that the genetic risk for PCOS is not associated with heel BMD and fracture risk in men.

This was a study done in a Caucasian population with no replication. However, this to date is the most extensive MR study looking at BMD and risk of fractures in women with a genetic predisposition to PCOS, whilst also providing some preliminary results in men. Like all the MR studies, our study is subject to weak instrument bias. *Another limitation of the study is that we included all self-reported all-cause fractures for the analysis to ensure adequate power. Further studies will be needed to ascertain if the protective effect of the genetic risk of hyperandrogenism in women living with PCOS is restricted to different types of traumatic and non-traumatic (fragility) fractures.* Despite the limitations this is the largest MR study looking at the effect of genetic risk of PCOS and BMD and risk of fractures in women and men and further studies in a diverse populations will be needed to confirm or refute these findings.

In summary, we show that the excess genetic risk for hyperandrogenism in women with PCOS is associated with a higher heel BMD and reduced risk of fractures. These data support the current guidelines which do not recommend routine monitoring of BMD and fracture risk in women with PCOS.

Data Availability Statement: The data underlying this article are available in the UK Biobank data

242 Acknowledgement: This research has been conducted using the UK Biobank Resource under
243 Application Number 44242 and 61479.

Table 1: Demographic characteristics of the study population .

Parameters	**Women**(n=221,086)	**Men** (n=187,816)
Age (years)	56.7±7.9	57.1±8.1
BMI (kg/m^2)	27.0 ±5.1	27.7±4.1
Heel BMD (g/cm^2)	0.50±0.11	0.56±0.12
Fractures n (%)	24,797 (11%)	17,076(10%)

Data are presented as mean±SD or n (%). BMD: bone mineral density, BMI: body mass index

Table 2: Association of a weighted genetic risk score for testosterone levels in PCOS with bone mineral density (BMD) and fractures

	Women		**Men**	
Phenotype	Effect Estimate	P-value	Effect Estimate	P-value
Heel BMD Beta (SE)*	0.0007 (±0.0002)	0.001	0.0002(±0.0002)	0.43
Fractures OR (95%CL)*	0.97 (0.96, 0.99)	0.003	1(0.99-1.02)	0.27

*Adjusted for Age, BMI and population stratification

Figure 1: Mendelian Randomisation model and the study hypothesis

Figure 1 legend: Mendelian randomization analysis

Figure 2a and 2b: Scatters plots showing the effects of PCOS associated SNPs with testostereone levels and BMD and fractures

Figure 2a legend: show scatter plots showing the associations of the PCOS associated SNP effects on the testosterone levels against the SNP effects on the BMD in the UKBiobank data.

Figure 2b legend: show scatter plots showing the associations of the PCOS associated SNP effects on the testosterone levels against the SNP effects on the fracture in UKBiobank data.

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