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BRIEF REPORT

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Implication of vasopressin receptor genes (*AVPR1A* and *AVPR1B*) in the susceptibility to polycystic ovary syndrome

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Abstract

Background Polycystic ovary syndrome (PCOS) is a complex heterogenous disorder manifesting with various reproductive, endocrine, and metabolic derangements such as insulin resistance and hyperglycemia. The arginine vasopressin peptide (AVP), also called or antidiuretic hormone (ADH), modulates metabolic functions such as glucose hemostasis, insulin sensitivity, and lipid metabolism via binding to two central and peripheral receptors (AVPR1A and AVPR1B). In the present study, we aimed to detect whether the *AVPR1A* and *AVPR1B* genes confer risk for PCOS.

Methods In peninsular Italian families, we tested 7 variants in the *AVPR1B* gene and 2 variants in the *AVPR1A* gene via Pseudomarker for linkage and linkage joint to association (i.e.., linkage disequilibrium) with PCOS.

Results We identified two risk variants in each gene, significantly associated with the risk of PCOS.

Conclusion To the best of our knowledge, this is the first study to report risk variants in *AVPR1A* and *AVPR1B* genes in association with PCOS. However, replication in other ethnic groups as well as functional studies are needed to confirm these results.

Keywords Polycystic ovary syndrome, Cortisol, Hypothalamic–pituitary–adrenal axis, Insulin resistance, Type 2 diabetes, Parametric analysis, Linkage disequilibrium, Single nucleotide polymorphisms, Arginine vasopressin receptor 1a, Arginine vasopressin receptor 1b

Introduction

Polycystic ovary syndrome (PCOS) is a complex heterogenous disorder manifesting with various metabolic, endocrine, and reproductive derangements [1]. Typical clinical features include signs of biochemical and/or clinical hyperandrogenism (e.g., elevated blood levels of testosterone, hirsutism and acne), signs of anovulation (e.g., oligoamenorrhea) and metabolic features (e.g., insulin resistance and obesity) [1, 2]. Several studies have shown that PCOS is associated with dysfunctional hypothalamic-pituitary–gonadal axis (HPG), including abnormal gonadotropin-releasing hormone (GnRH) pulse frequency, abnormal ovarian steroidogenesis and insulin resistance [3, 4]. Other studies have reported that the hypothalamic–pituitary–adrenal (HPA) axis is also impaired in PCOS [5]. We recently reported the association of corticotropin-releasing hormone receptor genes (*CRHR1* and *CRHR2*) with the risk of PCOS [6].

The peptide prohormone of arginine vasopressin (AVP) is synthesized in hypothalamic neurons and converted to AVP (also named antidiuretic hormone [ADH]), and has been studied for its roles in

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endocrine, metabolic, and neuropsychiatric pathways [7]. AVP moves across the axon of the posterior pituitary to be released into the blood in response to extracellular fluid hyperosmolality [7]. AVP affects the HPA axis by stimulating adrenocorticotropin hormone (ACTH) synthesis, which modulates adrenal steroidogenesis [8]. AVP also modulates metabolic functions such as glucose hemostasis and lipid metabolism [9]. In rodents, AVP neurons interact with GnRH neurons and form a part of the neural circuit implicated in PCOS [10]. There are 3 known AVP receptors: arginine vasopressin receptor 1a (AVPR1A), arginine vasopressin receptor 1b (AVPR1B) and arginine vasopressin receptor 2 (AVPR2) with relatively homologous amino acid sequences [8]. *AVPR1A* is expressed in the liver, adrenal gland and adipose tissue, while *AVPR1B* is predominantly expressed in the anterior pituitary [11] modulating the secretion of ACTH in various social behaviors which—if impaired—can lead to aggression, anxiety, and depression [12]. In animal studies, AVP was shown to enhance insulin sensitivity via AVPR1A receptor and to suppress sensitivity via AVPR1B receptors [8]. Of interest, the expression of *AVP* in the suprachiasmatic nucleus (SCN) is associated with the expression of *clock* genes in female rats, notably the HPG axis and circadian rhythm, which plays a key role in GnRH-secretion patterns [13].

PCOS exact pathophysiology is not yet understood [14]. Within the various endocrine, metabolic, and psychiatric dysfunctions of PCOS, the role of *AVPR1A* and *AVPR1B* expression in hypercortisolism, insulin resistance, and social behaviors is implicated in various studies [7, 14, 15], but further investigations are warranted. As *AVPR1A* and *AVPR1B* are expressed in multiple tissues and cells in the neuroendocrine, metabolic, and central nervous systems [11, 16], they could potentially play a role in the pathophysiology of PCOS. In this study, we aimed to investigate the implication of the *AVPR1A* and *AVPR1B* genes in the risk of PCOS.

Results and discussion

We identified 2 variants in the *AVPR1A* gene and 2 variants in the *AVPR1B* gene significantly associated with risk for PCOS in the Italian families ($P<0.05$) (Table 1). Two of the variants are missense (Figs. 1 and 2). The variants are mostly significant under the D1 model, indicating association at the allelic rather than genotypic level (Fig. 3). None of the variants has been previously associated with the risk of PCOS or any of its related phenotypes (namely, metabolic syndrome, hyperglycemia, irregular menses, anovulation, infertility, acne, oligomenorrhea, obesity, insulin resistance, T2D, hyperandrogenism, hirsutism).

Despite the studied families having been ascertained primarily for a T2D study, the *AVPR1A* and *AVPR1B* genes did not show statistically significant results with T2D. This is therefore, to the best of our knowledge, the first study to report risk variants in *AVPR1A* and *AVPR1B* genes in association with PCOS. The implication of these two receptor genes in PCOS could be potentially explained by their known roles in modulating insulin sensitivity [17] which is a pivotal pathogenic mechanism in PCOS [18]. Our *in-silico* analysis revealed that the 4 risk variants reported in our study intersect with globally repressed chromatin state and potential negative expression of *AVPR1A* and *AVPR1B* (RegulomDB [19]). The latter finding is consistent with the fact that concomitant knock-out of the two receptors in mice and rats causes impaired glucose tolerance [20, 21]. In addition, the two arginine vasopressin receptors could be potentially implicated in PCOS via modulating the feeding behavior and subsequent weight gain [22, 23]. Another potential mechanism is the mediated disruption of the sleep cycle [24] and circadian rhythm, both knowingly implicated in PCOS [25, 26] and in which AVPR1A has been implicated [27]. Interestingly, our *in-silico* analysis predicted that the *AVPR1B* PCOS-risk missense variant rs28632197 (p.Arg364Leu) affects the DNA-binding of the nuclear respiratory factor 1 (NRF-1) (Fig. 4), which is part of the

Table 1 Polycystic Ovarian Syndrome (PCOS) *AVPR1A* and *AVPR1B*-Risk Single Nucleotide Polymorphisms (SNPs)

Gene	Model[1]	SNP	Position	Ref	Alt	Risk Allele	Consequence	LD block	Reported in PCOS or associated phenotypes/traits*?
AVPR1A	D1, R1, R2	rs74097652	63,147,944	T	C	T	Intronic	Independent	Novel
	D1	rs113578517	63,149,913	G	T	T	Missense (F308L)	Independent	Novel
AVPR1B	D1	rs28632197	206,110,373	C	T	T	Missense (R364L)	Independent	Novel
	D2	rs3891058	206,116,219	A	G	G	Synonymous (H224=)	NA	Novel

[a] Models: D1: dominant, complete penetrance; D2: dominant, incomplete penetrance; R1: recessive, complete penetrance; R2: recessive, incomplete penetrance

* Phenotypes and traits associated with PCOS which were not reported: metabolic syndrome, hyperglycemia, irregular menses, anovulation, infertility, acne, oligomenorrhea, obesity, insulin resistance, T2D, hyperandrogenism, hirsutism

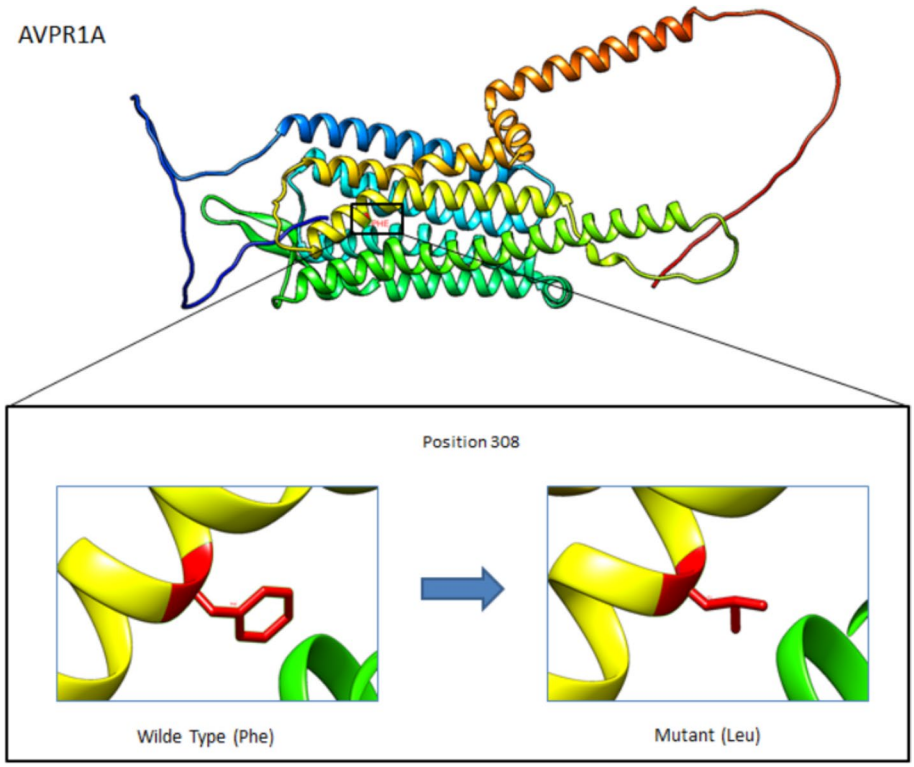

Fig. 1 3D structure of AVPR1A protein showing the position of the mutant missense risk variant (rs113578517) reported in our study

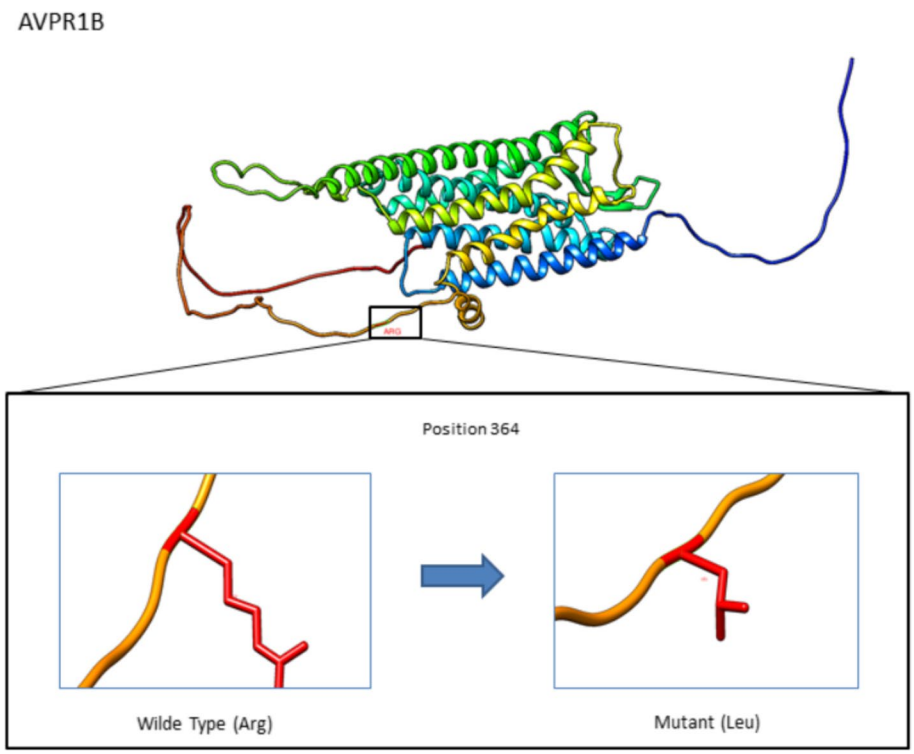

Fig. 2 3D structure of AVPR1B protein showing the position of the mutant missense risk variant (rs28632197) reported in our study

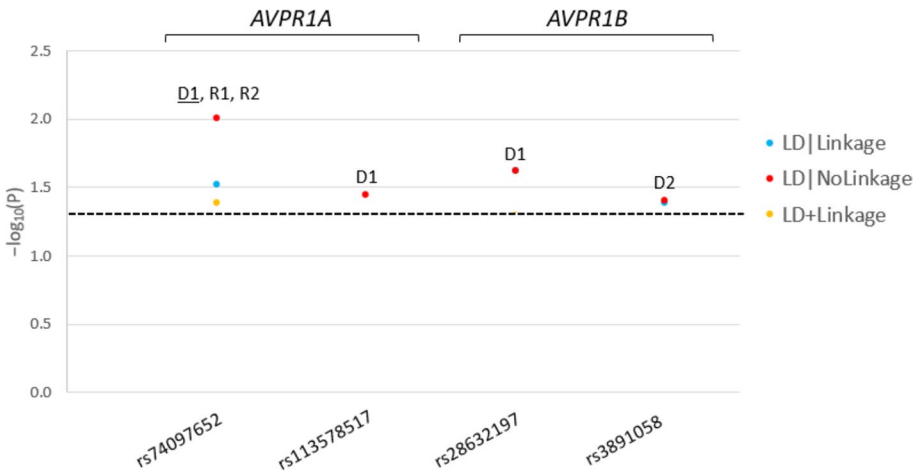

Fig. 3 Parametric Analysis Results of Polycystic Ovarian Syndrome (PCOS) AVPR1A and AVPR1B-Risk Single Nucleotide Polymorphisms (SNPs). For each AVPR1A and AVPR1B-risk SNPs in PCOS, we present the −log10(P) as a function of the significant ($p < 0.05$) test statistics [(linkage disequilibrium (LD)|Linkage, LD|No Linkage and LD + linkage] and per inheritance model. D1: dominant, complete penetrance, R1: recessive, complete penetrance, R2: recessive, incomplete penetrance. The most significant model is underlined.

circadian rhythm pathway disrupted in PCOS [28]. However, replication of our genetic finding in another ethnic cohort with PCOS and functional studies are needed to elucidate the pathogenic roles of *AVPR1A* and *AVPR1B* genes in PCOS.

Methods

Having recruited 212 Italian families for a prior T2D study [29–31], we re-investigated the same families for PCOS, phenotyped according to the Rotterdam diagnostic criteria (presence of at least two of the following three characteristics: chronic anovulation or oligomenorrhea, clinical or biological hyperandrogenism, and/or polycystic ovaries) [32]. Only Italian individuals of at least 3 generations and diagnosed with PCOS according to the above criteria and who were drug naïve were included in the study. Subjects were excluded if they were pregnant, of uncertain paternity, identical twins, or affected by primary amenorrhea, hypothalamic-hypogonadotropic amenorrhea, non-classical congenital

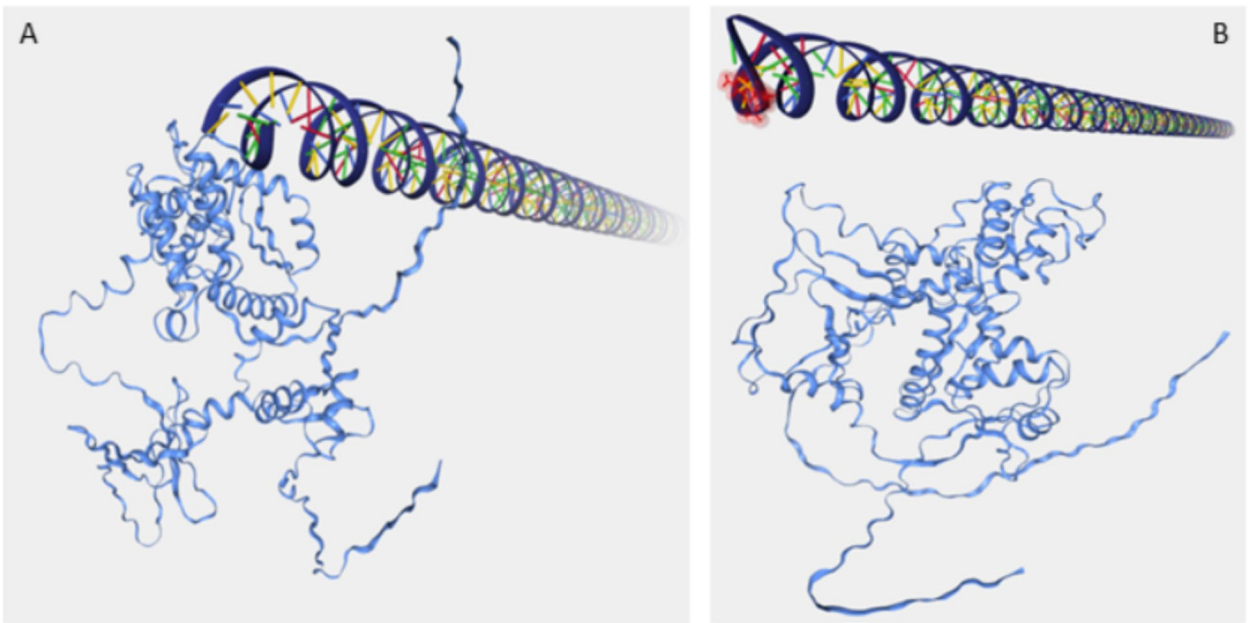

Fig. 4 3D illustration of the predicted binding of nuclear respiratory factor 1 (NRF-1) to a segment of AVPR1B gene (**A**) which is disrupted by the variant rs28632197 (C > T) shown in red (**B**)

adrenal hyperplasia, hyperprolactinemia, thyroid dysfunction, androgen-secreting tumor, hyperthecosis, or Cushing syndrome.

Within the 212 families with T2D (586 males, 573 females), 11% of families are positive for PCOS including 23 women with PCOS and 158 unaffected; the remaining 978 individuals, including males, labeled as unknown per phenotype, are disregarded by the Pseudomarker analysis. T2D is present in 73% of the subjects with PCOS, mostly treated by diet and/or oral medications. The patients with PCOS have an average maximum lifetime BMI = 32.51 (range 20.57–69.85) with 74% being at least overweight (BMI ≥ 25) and 39% being obese (BMI ≥ 30). Among the 158 individuals without PCOS, circa 88% have T2D (mostly treated by diet and/or oral medications) and the average maximum lifetime BMI is 30 (range 17.93–60.52) with circa 73% at least overweight with BMI ≥ 25 and 40% obese with BMI ≥ 30. We previously collected whole blood samples from individuals, from which DNA was extracted per the traditional phenol/chloroform method.

We investigated the linkage and linkage plus association (i.e., linkage disequilibrium [LD]) of 7 variants within the *AVPR1B* gene and 2 variants within the *AVPR1A* gene with PCOS according to the following models: dominant models with complete (D1) and incomplete penetrance (D2) and recessive model with complete penetrance (R1) and incomplete penetrance (R2). The variants were tested for both linkage and LD using the software Pseudomarker [33] after excluding Mendelian and genotyping errors via PLINK [34]. Specifically, we utilized familial genomic data from the UK Biobank Axiom Array platform, which had undergone rigorous quality control (QC, ≥ 0.96; SNPs to be considered valid had to reach a quality control of at least 0.96). We ran random replicates from the samples to verify the results' accuracy. We checked samples for kinship correlation verification. Furthermore, analysis via PLINK [34] was performed initially to exclude any Mendelian and genotyping errors, allowing to detect any potential adoption case, paternity uncertainty, or sample swap. The analyses we ran were free of any potential error. Familial studies offer an additional data quality verification step as the kinship correlation and the genotype assignment can be further verified via the inheritance within families. In addition, the software Pseudomarker offers a robust methodology to simultaneously test for linkage and LD in a combination of familial and singleton samples, exploiting the authentic pedigree relationships without depending on artificial assumptions to reveal statistical linkage effects [33].

Pseudomarker analysis output includes the test statistics LD|Linkage, LD|No Linkage and LD + linkage. Variants with $P < 0.05$ were considered significant. We also tested the amplified variants for the presence of LD blocks in the Tuscany population from the 1000 Genomes Project (https://www.internationalgenome.org/data-portal/population/TSI) and defined as "independent" the variants that are not within an LD block. The study adhered to the guidelines of the Helsinki Declaration and received approval from the Bios Ethical Committee (Prot.PR/Mg/Cg/311708). Written informed consent was obtained from each participant prior to the commencement of the study.

In-silico analysis

We conducted *in-silico* analysis to predict the risk variants-related disruption of transcription-factor binding (SNP2TFBS [35]), splicing (SNP-function prediction [36]), 3D protein structure (Chimera [37]) and miRNA binding (mirSNP [38]). The 3D modeling of DNA-binding protein was performed by HADDOCK2.2 [39].

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Institutional review board statement

Families were recruited following the Helsinki declaration guidelines, and individuals provided written informed consent prior to participation. The Bios Ethical Committee approved this study.

Authors' contributions

C.G. conceived and supervised the project, including statistical analysis and manuscript drafting. P.G. helped with the manuscript drafting and literature search. All authors have approved the final manuscript.

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Availability of data and materials

The study data are available on reasonable request, and due to lacking specific patients' consent and privacy restrictions, they are not publicly available.

Declarations

Competing interests

The authors declare no competing interests.

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