A homozygous mutation of *GNRHR* in a familial case diagnosed with polycystic ovary syndrome

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Abstract

Context: PCOS is a heterogeneous condition characterized by hyperandrogenism and chronic anovulation and affects about 10% of women. Its etiology is poorly known, but a dysregulation of gonadotropin secretion is one of its hallmarks.

Objective: As the etiology of PCOS is unclear, we have performed a genome-wide analysis of a consanguineous family with three sisters diagnosed with PCOS.

Methods: Whole-exome sequencing and Sanger sequencing confirmation.

Results: Whole-exome sequencing allowed the detection of the missense variant rs104893836 located in the first coding exon of the *GNRHR* gene and leading to the p.Gln106Arg (p.Q106R) substitution. Sanger sequencing of all available individuals of the family confirmed that the variant was homozygous in the three affected sisters and heterozygous in both parents.

Conclusions: This is the first description of a *GNRHR* gene mutation in patients diagnosed with PCOS. Although we do not exclude a possible interaction of the identified variant with the genetic background and/or the environment, our result suggests that genetic alterations in the hypothalamo–pituitary axis may play role in the pathogenesis of PCOS.

European Journal of
Endocrinology
(2017) **176**, K9–K14

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by hyperandrogenism and chronic anovulation. It affects up to 10% of women (1, 2) and at least 1 in 15 women of reproductive age, making this disorder the most common endocrine abnormality in this age group (3).

Mammalian reproduction depends on the activity of neurons able to secrete GnRH. These neurons send projections to the hypothalamus where GnRH is released into the pituitary portal system to reach the anterior pituitary and control the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone

(LH). Alterations of this system are associated with reproductive disorders such as hypogonadotropic hypogonadism (HH), hypothalamic amenorrhea and PCOS (4). Indeed, although the etiology of PCOS is poorly known, a dysregulation of gonadotropin secretion is one of its hallmarks. In patients with PCOS, plasma LH is often increased and FSH is often in the lower follicular range. This may be related to a decreased sensitivity of the GnRH pulse generator to inhibition by ovarian steroids, leading to enhanced LH secretion (5).

Various susceptibility genes involved in the TGF- β pathway, insulin signaling or associated with type 2

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diabetes and/or obesity have been tested for association with PCOS. Some of the most outstanding ones are fibrillin-3 (*FBN3*), insulin (*INS*), insulin receptor (*INSR*), insulin receptor substrate 1 (*IRS1*), transcription factor 7-like 2 (*TCF7L2*), calpain 10 (*CAPN10*), fat and obesity-associated gene (*FTO*) and sex hormone-binding globulin (*SHBG*) (5). Variants in the anti-Müllerian hormone (*AMH*) and its type II receptor (*AMHR2*) have also been suggested to play a role (6). However, although such PCOS susceptibility candidate genes have been detected with various degrees of certainty, we are still far from fully understanding the genetic contribution to this disorder.

Here, we report on a consanguineous family with three patients diagnosed with PCOS carrying a homozygous *GNRHR* mutation.

Patients and methods

Participants and data collection

We have performed a molecular analysis of the MO7 family in which three members had been diagnosed with PCOS. This family was recruited through the Genetic Institute of the HaEmek Medical Center in Afula (Israel). Blood samples were collected for hormonal and genetic studies. Genomic DNA isolation was performed by standard procedures. Written informed consent was obtained from all individuals included in the study, and the studies were performed according to the Declaration of Helsinki and approved by the local ethics committee.

MO7 is a consanguineous Middle East family of Arab origin. The parents (individuals IV-11 and IV-12, Fig. 1A) are first cousins and are connected by at least four other consanguineous marriages. They had 3 daughters diagnosed with PCOS (V-16, V-17 and V-18) and 2 sons.

The diagnosis of PCOS was done according to the Rotterdam criteria. All three sisters had oligomenorrhea, clinical hyperandrogenism and polycystic ovary morphology, suggesting a PCOS. The presence of acne was obvious and hirsutism was diagnosed subjectively (no Ferriman–Gallwey scoring available). However, as at least two criteria were fulfilled, the diagnosis of PCOS according to the Rotterdam criteria was proposed. Nonclassic congenital adrenal hyperplasia, hyperprolactinemia and hypothyroidism were ruled out by the relevant tests (17-hydroxyprogesterone: range for the examined patients 0.66–1.9 ng/mL (normal range 0.2–5 ng/mL); prolactin: range for the examined patients 8.55–20.32 ng/mL (normal range 2–20 ng/mL); TSH: 1.79–4.2 IU/L (normal range 0.5–5 IU/L)). Normal gonadotropin levels

ruled out ovarian failure and classical HH (LH, range for the examined patients: 4.2–10.3 IU/L (normal range, follicular phase 2–15 IU/L); FSH, range for the examined patients: 3.07–6.6 IU/L (normal range, follicular phase 3–20 IU/L)). All these elements taken together pointed to a diagnosis of PCOS. All three sisters consulted for infertility but conceived either spontaneously or after fertility treatment.

Whole-exome sequencing and variant analysis

To investigate a potential underlying genetic cause, we performed a whole-exome sequencing of two PCOS patients V-17 and V-18 and of their unaffected mother IV-12. Library preparation, exome capture, sequencing and data processing were performed by IntegraGen SA (Evry, France) using standard procedures (7). Genomic copy number variants (CNV) were assessed using the Bioconductor DNACopy package (DNACopy 1.32.0) by comparing the exome data to reference samples. Whole-exome sequencing generated 55 to 103 million mapped read pairs per sample and covered 96% of the targeted sequence at 10× or greater. Consanguinity in this family strongly suggested an autosomal recessive inheritance of a disease allele from one of their common ancestors. We filtered the single nucleotide variants (SNV) and indels according to the following criteria: minimum depth of 5 reads, location in the coding sequences with potential impact on transcript or protein (i.e. non-synonymous changes), homozygosity in both affected sisters and heterozygosity in the mother. The minor allele frequency (MAF) was set to be under 1% when the variant was present in the public variant databases dbSNP132, 1000 Genomes Project (release_v3 20101123), Exome Variant Server/EVS (ESP6500SI-V2-SSA137), HapMap3, ExAc and in an IntegraGen internal database (containing 201 control exomes for SNPs and 130 control exomes for indels). The variants were filtered using IntegraGen's ERIS platform, VEP and VarSifter 1.7. We further refined the list of variants by keeping only those predicted as probably damaging and deleterious according to Polyphen, SIFT and Condel.

Results and discussion

The etiology of the syndrome is not completely understood yet; however, PCOS is considered to be a multifactorial disorder with various genetic, endocrine and environmental contributing factors. The presence of

Figure 1

Analysis of the consanguineous MO7 family. (A) Pedigree of the MO7 family. Squares and circles indicate males and females respectively. Black circles represent females affected with PCOS. Double lines connect individuals involved in consanguineous unions. The blue arrows indicate the three individuals analyzed by whole-exome sequencing. The genotype for the variant position in *GNRHR* is indicated below each individual (as shown by Sanger sequencing). T, wild-type allele; C, mutated allele. (B) Visualization of whole-exome sequencing data in IGV around the *GNRHR* variant. The high coverage (i.e., total read count above 200) and the variant counts show that the two affected sisters and the unaffected mother are respectively homozygous and heterozygous for the variant. (C) Validation of the missense variant by Sanger sequencing. The three affected sisters were confirmed to be homozygous for the Q106R variant by Sanger sequencing (typical chromatogram), using the primers GNRHR-Ex1 Forward (5' AGATCCGAGTGACGGTTACTTT-3') and GNRHR-Ex1 Reverse (5'-CTGTCCGACTTTGCTGTTGCTT-3'). (D) Schematic representation of the human *GNRHR* gene and protein. The positions of the substitutions (at DNA and protein levels) are shown.

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three affected sisters in the consanguineous MO7 family led us to explore a potential genetic etiology.

First of all, despite a high coverage in the exome data, we could not identify any candidate variants in *INS*, *INSR*, *IRS1*, *TCF7L2*, *CAPN10*, *FTO*, *SHBG*, *AMH* or *AMHR2*, which have previously been tested for association with PCOS. In *FBN3*, one missense variant (p.D911V) with a MAF <1% was found heterozygous in the two PCOS patients and not present in the unaffected mother (Supplementary Table 1, see section on supplementary data given at the end of this article). Nevertheless, the association of *FBN3* variants and PCOS is still unclear (8), and our study is not designed to formally demonstrate the involvement of such variants. We also checked exome data for co-segregation of CNVs with the phenotype and identified a duplication involving the *UGT2B17* and *UGT2B15* genes, whose gene products are known to be implicated in the excretion of steroid hormones (9). Although potentially interesting, gains or losses of these two genes are frequent, according to the DGV database (<http://dgv.tcag.ca/dgv/app/home>) and their influence on PCOS can only be assessed by an association study. Below, we discuss a much more compelling candidate variant.

Amid the 2 SNVs that passed all filters of exome sequencing analysis described in the 'Patients and methods' section, one was already known as giving rise to a pathogenic allele in the context of human reproduction. This missense variant chr4:68619737 T>C (rs104893836) is located in the first coding exon of the *GNRHR* gene and leads to the p.Q106R substitution (Fig. 1B). The other variant was 4:1164836 G>A leading to the missense mutation Thr196Ile in Spondin 2 (*SPON2*). This protein plays a role in cell invasion, migration and hepatoma progression (10) but has no known link with reproduction or reproductive disorders. Although we cannot exclude a potential impact of this variant on PCOS, we focused on the *GNRHR* gene variant, which has clearly been incriminated in other reproductive phenotypes. Sanger sequencing of all available individuals of the family confirmed that the rs104893836 (p.Q106R) variant in *GNRHR* was homozygous in the three affected sisters and heterozygous in both parents (healthy carriers) (Fig. 1B and C).

The *GNRHR* gene encodes the gonadotropin-releasing hormone receptor, which is a G protein-coupled receptor displayed at the surface of pituitary gonadotrope cells. After binding of GNRH, the receptor interacts with G-proteins that activate the phosphatidylinositol-calcium second messenger system. This causes the release of LH and FSH. p.Q106R is a rather commonly occurring variant

of *GNRHR* that affects its first extracellular loop (Fig. 1D). It reaches an allelic frequency of 0.4% in European populations and 0.25% in general. Interestingly, this Q106R variant was shown to be included in a common haplotype consistent with a founder event (11). However, the variant rs104893836 in the MO7 family does not appear to lie within this founder haplotype and is likely to have appeared independently.

The p.Q106R was the first *GNRHR* variant to be identified, in compound heterozygosity, in a patient with partial HH (12). Functional analyses of the p.Q106R variant have revealed a significantly reduced GNRH binding. It also results in a decrease in phospholipase C activity and intracellular inositol-phosphate production in response to GNRH (12, 13). However, despite this partial loss of function observed *in vitro*, p.Q106R seems to have mild effects *in vivo* (4). This variant appears most of the time in compound heterozygosity. However, three unrelated patients, two males and one female, have been reported to be homozygous for Q106R (14). All three patients display a mild phenotype, with a partial sexual development and for two of them, a spontaneous reversal of HH was reported. One male displayed a fertile eunuch syndrome (15) and the other was reported as eunuchoid (16). The 27-year-old female patient was reported as affected with partial idiopathic HH and achieved a spontaneous pregnancy 3 months after oral contraceptive pill withdrawal. In this female patient, basal levels of FSH and LH were within the normal range, as is the case for the patients we describe. Interestingly, three months after contraceptive pill withdrawal, the patient was amenorrheic, had high beta-hCG serum level and transvaginal ultrasound revealed the presence of two embryos. It seems that contraceptive withdrawal induced a transient situation of endogenous pulsatile GNRH secretion, overcoming the partial resistance due to the Q106R mutation, thus allowing ovulation (17).

PCOS is associated with altered GNRH pulses, an excess of LH and insufficient FSH secretion, which contribute to excessive ovarian androgen production and ovulatory dysfunction (1). It is not clear yet whether the alteration of the hypothalamo-pituitary axis in PCOS is primary or secondary to alterations in steroid hormones secretion (2). Interestingly, the p.Q106R variant has been shown to reduce the sensitivity of the FSH gene promoter to GNRH to a greater extent than that of LH (4).

The link between *GNRHR* variants and PCOS might not appear straightforward at first sight. Indeed, in women with PCOS, it has been proposed that differences in GNRH pulsatility might also be due to a decreased

sensitivity to progesterone feedback in the presence of normal *GNRHR* receptivity (18). For the moment, it is unclear how a *GNRHR* defect would fit in this picture. However, a previous work has described a group of HH patients with ultrasound evidence for PCOS, in which the ovarian response to ovulation induction was similar to that of patients with PCOS (19). It is possible that in the family described here, two doses of the variant p.Q106R led to (at most) a subclinical HH associated with a PCO-like syndrome.

Obviously, our findings need to be replicated through additional mutation screening of the *GNRHR* gene in patients with PCOS. That said, we expect that this variant would be a minor cause of PCOS. Indeed, if we consider that the (conservative) allelic frequency of the variant is 0.4% (0.004), a homozygous individual (male or female) is expected at a frequency of 1.6×10^{-5} . The frequency of homozygous females in reproductive age would be $1.6 \times 10^{-5} \times 0.5$ (proportion of females) $\times 0.5$ (proportion of females in reproductive age) = 4.10^{-6} . Even though PCOS is frequent in such women, this figure implies that the screening of a very large PCOS cohort (>1000 women) is necessary to find another homozygous individual and also to assess the potential impact of co-occurring variants, such as the SNV in *FBN3* or the CNV involving *UGT2B15* and *UGT2B15*, previously mentioned.

To the best of our knowledge, this is the first description of a *GNRHR* gene mutation in three patients diagnosed with PCOS. Although we cannot exclude a possible interaction between the identified variant and the genetic background or the environment, our result suggests that genetic alterations in the hypothalamo-pituitary axis may play a role in the pathogenesis of PCOS.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-16-0968>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this case report.

Funding

This study was supported by Université Paris Diderot, the Fondation pour la Recherche Médicale (grant DEQ20150331757) and the Fondation Maladies Rares ('High throughput sequencing and rare diseases' research program PJ1502024).

Author contribution statement

S C, R B F, M F, S S and R A V designed the study. S C and R A V analyzed exome data. B L performed Sanger sequencing. S C, R B F, M F, S S, B L

and R A V wrote the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

The authors thank IntegraGen for being so efficient and kindly helpful. They thank three anonymous referees for their most helpful comments.

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Received 24 November 2016

Revised version received 23 January 2017

Accepted 30 January 2017